

Breastmilk Composition of HIV-Infected Mothers Receiving Antiretroviral Therapy who gave birth to Premature Infants

by
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DECLARATION

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ABSTRACT

Background

The incidence of premature birth is rising in Southern Africa. Premature birth is associated with, among other, human immunodeficiency virus (HIV)-infection during pregnancy. Women with HIV, chronic malnutrition and obesity are more likely to give birth to premature infants with intra-uterine growth restriction (IUGR). Providing the HIV-exposed premature infant with breastmilk accompanied by maternal and infantile anti-retroviral therapy (ART) are key strategies to reduce HIV mother-to-child-transmission (MTCT), and infant morbidity and - mortality. Recent literature showed a difference in the breastmilk composition of HIV-infected and HIV-uninfected mothers. The effects of HIV infection and ART on the breastmilk composition of mothers of premature infants are, however, largely unknown.

Aims and Objectives

The main aim of the study was to assess and compare the breastmilk composition of HIV-infected mothers receiving ART and HIV-uninfected mothers who gave birth to premature infants. Secondary objectives of the study were to assess the maternal nutritional status of HIV-infected and HIV-uninfected mothers as well as to assess the neonatal nutritional status of premature infants in relation to maternal nutritional status, HIV status and ART regimen.

Participants and Methods

The study was designed as a cross-sectional, descriptive study with an analytical component. Study participants included HIV-infected and HIV-uninfected mothers who gave birth to premature infants. The women were subdivided into four groups according to HIV-status and the length of gestation. Mothers provided demographic information and two breastmilk samples on day seven and nine of lactation. Maternal anthropometric data [weight, height and mid-upper arm circumference (MUAC)] were collected on day seven postpartum. Infant anthropometric data [weight, length and head circumference (HC)] were obtained at birth and length and HC data were obtained once more on day seven

postpartum. Breastmilk samples were analysed for energy, protein, carbohydrates, fat, phosphate, iron, zinc and copper.

Results

The study population consisted of 38 HIV-infected women receiving ART and 36 HIV-uninfected women who gave birth to premature infants. Protein (1.95 vs. 1.78 g/100g; $p=0.04$), fat (4.42 vs. 3.49 g/100g; $p=0.01$) and copper (0.64 vs. 0.56 mg/l; $p=0.02$) in breastmilk samples were higher while carbohydrate (5.37 vs. 6.67 g/100g; $p=0.002$) and zinc (5.26 vs. 5.78 mg/l; $p=0.04$) were lower in HIV-infected women compared to HIV-uninfected women. Zinc levels were significantly lower in HIV-infected women with early gestation infants, with lowest levels in women who received ≤ 4 weeks ART (0.58mg/l; $p=0.03$). Total energy (78.22 vs. 61.48 kCal/100ml) and fat (5.39 vs. 3.00g/100ml) levels were significantly higher in late gestational HIV-infected women who received <4 weeks ART. Copper levels (0.61mg/l) were higher in late gestation women who received 4-20 weeks ART exposure ($p=0.05$). The variances in nutritive values in these milk samples did not, however, range outside the normal values of premature breastmilk composition.

The mean maternal BMI was 26.7kg/m² and MUAC was 289mm. Maternal undernutrition (9%) and obesity prevalence (9%) was low. There was a high prevalence of IUGR (54%). Neither maternal nutritional status ($p=0.79$) nor HIV-status and ART regimen ($p=0.72$) were associated with IUGR. Similarly, the nature of IUGR (symmetrical vs asymmetrical) was not associated with maternal HIV-status ($p=1.00$). Head circumference restriction was less prevalent in infants born to women with ART exposure >20 weeks ($p=0.003$).

Conclusion

HIV-infected women on ART can safely breastfeed their premature infants. Maternal nutritional status, HIV-status and ART regime did not influence neonatal nutritional status among premature infants in this study. Maternal ART over a longer period may protect the baby against IUGR, with specific reference to head circumference.

OPSOMMING

Agtergrond

Die voorkoms van premature geboortes is aan die toeneem in Suider Afrika. Daar is 'n beduidende verwantskap tussen premature geboorte en menslike immuniteitsgebreksvirus (MIV)-infeksie tydens swangerskap. Moeders met MIV of moeders wat chronies wangevoed is of vetsugtig is, is meer geneig om geboorte aan 'n premature baba met intra-uteriene groeivertraging (IUGV) te skenk. Twee belangrike strategieë om MIV-oordrag van moeder na kind te voorkom asook om morbiditeit en mortaliteit te verlaag is om premature babas met borsmelk te voed en terselfdertyd die moeder en baba van antiretrovirale terapie (ART) te voorsien. Onlangse literatuur rapporteer dat daar verskille in die borsmelksamestelling van MIV-geïnfekteerde en MIV-ongeïnfekteerde moeders is. Die uitwerking van MIV en ART op die borsmelksamestelling van die moeders van premature babas is egter tans onbekend.

Studiedoelwitte

Die primêre doel van die studie was om die borsmelksamestelling van moeders wat met MIV-geïnfekteer is en dié van moeders wat nie met MIV-geïnfekteer is nie, te bepaal en te vergelyk. Die sekondêre doelwitte was om die voedingstatus van MIV-geïnfekteerde en MIV-ongeïnfekteerde moeders te bepaal en te vergelyk, sowel as om die neonatale voedingstatus van premature babas te bepaal en in verhouding met maternale voedingstatus, MIV-status en ART-regime te evalueer.

Deelnemers en Metodes

Die studie-ontwerp was 'n beskrywende deursnit-studie met 'n analitiese komponent. Deelnemers het MIV-geïnfekteerde en MIV-ongeïnfekteerde moeders wat geboorte aan 'n premature baba geskenk het, ingesluit. Moeders is verder in vier groepe volgens MIV-status, gestasietydperk en ART-tydperk verdeel. Moeders het demografiese inligting asook twee borsmelkmonsters op dag sewe en nege van laktasie verskaf. Maternale antropometrie (gewig, lengte en midarmomtrek) is op dag sewe postpartum geneem. Neonatale antropometrie (gewig, lengte en kopomtrek) is met geboorte geneem en lengte en

kopomtrek is op dag sewe postpartum weer geneem. Die borsmelkmonsters is vir totale energie, proteïene, koolhidrate, vet, fosfaat, yster, sink en koper geanaliseer.

Resultate

Die studiepopulasie het uit 38 MIV-geïnfekteerde moeders wat ART ontvang en 36 MIV-ongeïnfekteerde moeders met premature babas, bestaan. Die proteïene- (1.95 vs. 1.78 g/100g; $p=0.04$), vet- (4.42 vs. 3.49 g/100g; $p=0.01$) en koperinhoud (0.64 vs. 0.56 mg/l; $p=0.02$) in die borsmelkmonsters was verhoog en die koolhidraat- (5.37 vs. 6.67 g/100g; $p=0.002$) en sinkinhoud (5.26 vs. 5.78 mg/l; $p=0.04$) was verlaag onder MIV-geïnfekteerde moeders vergeleke met dié van MIV-ongeïnfekteerde moeders. Sinkvlakke was beduidend laer onder MIV-geïnfekteerde moeders, met die laagste vlakke onder vroeë-gestase moeders met ART blootstelling <4 weke (0.58mg/l; $p=0.03$). Die totale energie- (78.22 vs. 61.48 kKal/100ml, $p=0.03$) en vetvlakke (5.39 vs. 3.00g/100ml, $p=0.04$) was beduidend hoër onder laat-gestase MIV-geïnfekteerde moeders met <4 weke ART. Kopervlakke was hoër (0.8mg/100ml) onder die laat-gestase MIV-geïnfekteerde moeders met 4-20 weke ART blootstelling ($p=0.05$).

Die gemiddelde maternale LMI was 26.7 kg/m^2 en die gemiddelde midarmomtrek was 289 mm. Die voorkoms van maternale ondervoeding (9%) en vetsugtigheid (9%) was laag. Daar was 'n hoë voorkoms van IUGV (54%). Maternale voedingstatus ($p=0.79$), MIV-status en ART-regime ($p=0.82$) was nie geassosieer met IUGV nie. Net so was die tipe IUGV (simmetries teenoor onsimmetries) nie geassosieer met maternale MIV-status nie ($p=1.00$). Kopomtrekvertraging was beduidend laer onder babas van moeders met >20 weke ART blootstelling ($p=0.003$).

Gevolgtrekking

MIV-geïnfekteerde moeders kan veilig hul premature babas borsvoed. Maternale voedingstatus, MIV-status en ART regime het nie IUGV in premature babas in hierdie studie beïnvloed nie. 'n Langer tydperk van ART blootstelling mag die baba teen kopomtrekvertraging beskerm.

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CONTRIBUTIONS

Carike Fouché (principal researcher), Evette van Niekerk and Lianne du Plessis (supervisors) designed the research study. Carike Fouché performed data collection and analysed the data. Carike Fouché, Evette van Niekerk and Lianne du Plessis drafted the manuscripts and reviewed the data; Suzanne Delport (supervisor) critically reviewed the papers. All authors read and approved the final versions of the manuscripts.

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LIST OF ABBREVIATIONS

AA:	Arachidonic Acid
ABER:	Auditory Brainstem Evoked Response
ALA:	Alpha Linolenic Acid
ART:	Anti-retroviral Therapy
ARV:	Anti-retroviral
DHA:	Docosahexanoic Acid
FDC:	Fixed Dose Combination
HAART:	Highly Active Anti-retroviral Therapy
HCT:	HIV Counseling and Testing
HDL:	High Density Lipoproteins
HIV:	Human Immunodeficiency Virus
HMO:	Human Milk Oligosaccharides
HPLC:	High Performance Liquid Chromatography
ICU:	Intensive Care Unit
IQ:	Intelligence Quotient
ISO:	International Standards Organisation
IUGR:	Intra-uterine Growth Restriction
KMC:	Kangaroo Mother Care
LA:	Linoleic Acid
LDL:	Low Density Lipoproteins
LBW:	Low Birth Weight
LC-PUFA:	Long-Chain Polyunsaturated Fatty Acids
MCT:	Medium-Chain Triglycerides

MTCT:	Mother-to-child transmission
MUAC:	Mid-upper arm circumference
NEC:	Necrotizing Enterocolitis
NNRTI:	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI:	Nucleoside Reverse Transcriptase Inhibitors
PI:	Ponderal Index
PMTCT:	Prevention of Mother-to-child Transmission
PUFA:	Polyunsaturated Fatty Acids
SGA:	Small for Gestational Age
VLBW:	Very Low Birth Weight
WHO:	World Health Organisation

BRIEF OUTLINE OF THE THESIS

This thesis is divided into four chapters. The outline of each chapter is as follows:

Chapter 1 covers the literature background of the investigation.

Chapter 2 describes the methodology of the investigation.

Chapter 3 presents the results of the investigation in two separate scientific articles.

Article 1: Differences in Breastmilk Composition of HIV-infected and HIV-uninfected mothers of Premature Infants: Effects of Antiretroviral Therapy.

Article 2: Anthropometric Parameters of HIV-Infected and HIV-Non Infected Mothers and their Premature Infants.

Chapter 4 concludes the study objectives and findings. A set of null hypotheses are accepted or rejected. Limitations of the study are discussed and recommendations for further research are made.

CHAPTER 1:

Literature Review

LITERATURE REVIEW

1.1 INTRODUCTION

A premature infant is defined as an infant born <37 weeks' gestation.¹ Fifteen million premature births occur worldwide of which 60% of these deliveries occur in low-and middle income countries.² Premature delivery is considered a significant global perinatal health problem with a rising incidence in Southern Africa. In 2010, the incidence of premature births was 17.5% compared to 6.2% and 10.6% in Europe and North America respectively.³ In a meta-analysis by Beck and colleagues, the worldwide prevalence of premature births was found to be 9.6% with 85% of these preterm births concentrated in Africa and Asia.³ Data from developing countries suggest that human immunodeficiency virus (HIV)-infected mothers have an increased risk of giving birth to premature infants and the occurrence is directly associated with clinical stage of disease.⁴⁻⁶ HIV-infected women were also found to be more likely to give birth to low birth weight (LBW) and growth restricted infants.⁴ A recent African study found the incidence of prematurity among HIV-infected mothers to be 19.1% and the incidence of LBW infants to be 11.5%.⁷

Premature infants have a substantially higher risk of dying and several short- and long-term complications due to immaturity of major organ systems.^{3,4} One of the key strategies to increase the survival rate of premature infants is to provide breastmilk early and exclusively. Breastmilk is the optimal feeding modality and is associated with lower rates of mortality and morbidity in the premature infant population. Furthermore, breastmilk provides the ideal nutritional, gastrointestinal and immunological components and is associated with psychological and developmental advantages in premature infants.⁸ Recent South African (SA) data showed that there were differences in certain compositional factors of HIV-infected and HIV-uninfected women who had given birth to term-⁹ and preterm infants.¹⁰

1.2 INTERVENTIONS THAT PROVIDE PROTECTION AGAINST MOTHER TO CHILD TRANSMISSION OF HUMAN IMMUNODEFICIENT VIRUS

Data from 2008 indicated that 10.9% of South Africans over the age of two years lived with HIV infection¹¹ and one in three SA mothers who attended antenatal clinics was HIV-infected.¹²

Given the seriousness of the SA HIV epidemic, concerns have been raised regarding the vertical transmission of HIV through breastmilk.¹³ In the past SA guidelines provided HIV-infected mothers with the option of either breastfeeding exclusively or providing formula milk exclusively. However, increased mortality rates due to non-compliance with these guidelines led to drastic policy changes.¹⁴ Consequently the World Health Organisation (WHO) feeding guidelines in the HIV context for developing countries, in other words providing mothers with one message on infant and young child feeding (IYCF), in this case exclusive breastfeeding and antiretroviral therapy (ART) for mothers and infants, were accepted.¹⁵

ART is an effective way of reducing the viral load, increasing the maternal CD₄ count¹⁶ and minimising the postnatal HIV mother-to-child-transmission (MTCT) risk through breastmilk.¹⁷ In 2006, the WHO released a prevention of mother-to-child- transmission (PMTCT) of HIV consensus statement that elicited guidelines on the provision of ART to mother and infant pairs as well as the appropriate feeding guidelines.^{18,19} South Africa accepted these guidelines in August 2011 and they were implemented in April 2012. The guidelines include the provision of ART to pregnant and lactating mothers who needed anti-retroviral management, and provided their infants with prophylactic ART treatment. In addition, exclusive breastfeeding is recommended from birth until six months and thereafter breastfeeding should be accompanied by the introduction of appropriate complementary foods.¹⁹⁻²¹ Continued breastfeeding until 12 months of age is recommended.²¹ This ART and IYCF strategy has been shown to be highly effective in preventing MTCT of HIV.¹⁸

The following sections provide a brief description of the current ART product description, ART regimes and the associated complications with the use of treatment.

1.3 ANTIRETROVIRAL THERAPY PRODUCT DESCRIPTION

Drug selection is a multifaceted process with six major anti-retroviral (ARV) drug classes and 25 drugs:

- **Nucleoside/nucleotide backbones:** These are considered the pillar of ART and are provided in pairs:

- **Nucleoside analogues/ nucleoside reverse transcriptase inhibitors (NRTI):** These interfere with DNA synthesis of HIV by incorporating defective building blocks into HIV DNA, preventing chain completion. The site of action is indicated by a green arrow in Figure 1.1. Drugs include: tenofovir and emtricitabine; abacavir and lamivudine or lamivudine and zidovudine.^{21,22}
- **Non-nucleoside reverse transcriptase inhibitors (NNRTI):** These interfere with reverse transcriptase enzyme's ability to convert the HIV RNA to HIV DNA. The site of action is indicated by a yellow arrow in Figure 1.1. Drugs include: efavirenz and nevirapine.^{21, 22}
- **Protease inhibitors (PI):** These oppose the protease enzyme that HIV uses to produce infectious viral components. The site of action is indicated by a blue arrow in Figure 1.1. Drugs include: atazanavir and darunavir.^{21, 22}

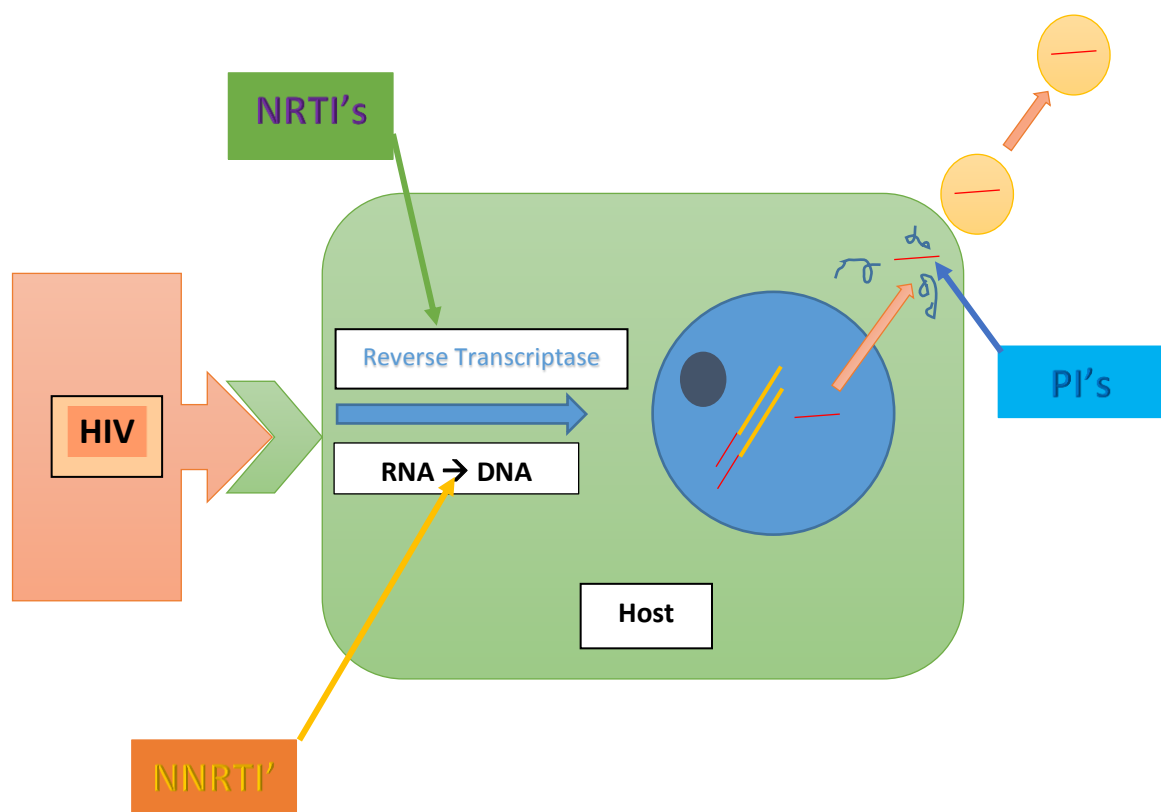


Figure 1.1: Major antiretroviral classes. Source: Adapted from University of Washington's classification of ART classes. Available from:

[http://www.chartcaribbean.org/careofplwa/pdfdocuments/subpdf/Section%20IV%20new/I-V-2 \(classes%20and%20characteristics\).pdf](http://www.chartcaribbean.org/careofplwa/pdfdocuments/subpdf/Section%20IV%20new/I-V-2%20(classes%20and%20characteristics).pdf).²²

1.4 CURRENT SOUTH AFRICAN ARV REGIMENS

In 2013, fixed dose combination (FDC) therapy, a one-pill combination of tenofovir, emtricitabine and efavirenz, was introduced as first-line treatment. In 2014, the eligibility criteria for pregnant women for the initiation of ART were increased from a CD₄ count of ≤ 350 to a CD₄ count of 500 cells/mm³ and the PMTCT programme adopted a B+ method that entitled all pregnant and lactating women to lifelong ART regardless of HIV status or stage of disease. Pregnant and lactating women who initially test HIV negative should have the test repeated after and then every three months throughout the duration of pregnancy and lactation as 4% of all women test positive three months after the initial test.²⁴

1.4.1 Adverse Metabolic Effects of Human Immunodeficiency Virus Treatment Schedules

The introduction of highly active antiretroviral therapy (HAART) in the 1990s was a huge advancement in the treatment of HIV by suppressing HIV RNA replication and significantly reducing HIV-related morbidity and mortality.²⁴

There is biochemical and clinical proof that HAART may induce changes in biochemical and metabolic profiles such as glucose and lipid profiles, bone metabolism and endocrine abnormalities.^{25,26} These potential changes are depicted in Table 1.1.

Table 1.1: Highly active antiretroviral therapy (HAART)-related metabolic abnormalities

HAART-related effects	
Lipodystrophy	<ul style="list-style-type: none"> ▪ Lipoatrophy ▪ Lipohypertrophy (fat accumulation)
Dyslipidaemia	<ul style="list-style-type: none"> ▪ ↑ Triglycerides ▪ ↓ High density lipoprotein (HDL) cholesterol ▪ ↑ Total and low density lipoprotein (LDL) cholesterol ▪ ↑ Hepatic de novo lipogenesis ▪ ↑ Small, dense LDL particles
Glucose tolerance and insulin resistance	<ul style="list-style-type: none"> ▪ ↓ Glucose tolerance ▪ ↑ Fasting insulin and proinsulin ▪ ↑ Proinsulin: insulin ratio ▪ Increased C-peptide
Endocrine	<ul style="list-style-type: none"> ▪ Bone and mineral: osteopenia and osteoporosis ▪ Thyroid: hypothyroidism ▪ Adrenal: mineralcorticoid, glucocorticoid or adrenal androgen dysfunction ▪ Pituitary: hypercorticosolaemia or glucocorticoid resistance ▪ Glucose intolerance

Source: Adapted from Anuurad et al.²⁵

1.4.2 Highly Active Antiretroviral Therapy (HAART)-Associated Lipodystrophy

With the introduction of ART, the clinical picture of the HIV-infected person changed from emaciation to body shape abnormalities and morphological features associated with glucose intolerance and lipid disturbances, such as central obesity and peripheral fat loss.²⁷⁻²⁹ The syndrome, 'HIV lipodystrophy', was coined. However, it soon became evident that it is not a single syndrome, because some patients present with lipoatrophy only, others with central fat accumulation and others with both.³⁰ The prevalence of HIV lipodystrophy ranges from 10 to 80% according to different definitions used. One should view lipoatrophy and fat

accumulation as two separate syndromes as both are associated with different mechanisms, risk factors and ART schedules.²⁹

Lipoatrophy is the loss of subcutaneous fat in the face (buccal and temporal region), extremities, abdomen and buttocks. In contrast to HIV-wasting syndrome, HIV-associated lipoatrophy is associated with subcutaneous fat loss with preservation of the lean muscle tissue.³⁰ Evidence shows that NRTIs, specifically the thymidine analogues- stavudine and zidovudine, are responsible for metabolic changes related to lipoatrophy. Studies also indicated that efavirenz minimally contributes to lipoatrophy.³² These changes are a result of NRTI-induced inhibition of mitochondrial DNA polymerase gamma and mitochondrial toxicity.³³ PI's have not been shown to induce lipoatrophy.³⁴

Fat accumulation (lipohypertrophy) is the result of a metabolic abnormality that causes surplus fat accumulation in the trunk, dorsal area and abdomen (central obesity). There may also be fat deposition in the liver, muscles, myocardium and epicardium. It is suggested that a defect in peripheral adipocytes causes an increase in the circulation of free fatty acids. The latter are then deposited in the visceral adipose tissue due to higher lipid turnover and – uptake.²⁶ ART-related hypertrophy has not been linked to a specific ART agent or class, but relates more to the host's risk factors.²⁹ Initially it was believed that PI ARV drugs caused fat accumulation; however, patients who had never received PI antiretroviral drugs also showed signs of fat accumulation.²⁵ In addition, numerous longitudinal studies failed to show an association between PI's and lipohypertrophy.^{26, 30} Risk factors for fat deposition include: advanced age, female sex, elevated baseline triglycerides and elevated body fat percentage.³⁵ There is also evidence to show that the fat accumulation in HIV-infected patients on ART may reflect the global obesity epidemic rather than the effects of HIV infection or ART use alone.³¹ However, the Multicentre AIDS Cohort Study illustrated that HIV-infected men had a faster increase in waist circumference and advanced size of dorsal fat pad (buffalo hump) than HIV-uninfected men.³⁶ Some studies have also shown that the quality of the diet of HIV-infected individuals is poor and relates to increased visceral fat deposition.³⁷

Furthermore, HAART-associated lipodystrophy causes a reduced expression of LDL that results in increased serum levels of LDL.²⁶ A decrease in HDL and triglycerides is also observed. These metabolic abnormalities are similar to those of patients with metabolic

syndrome. Changes are evident in advanced HIV disease as well as in asymptomatic HIV-infected patients.²⁵

Mitochondrial changes are also evident in HAART-associated lipodystrophy. Inhibition of mitochondrial DNA-polymerase γ causes mitochondrial DNA exhaustion in respiratory chain dysfunction that ultimately leads to reduced energy production in cells. The latter promotes metabolic disorders in adipocytes, lipodystrophy and increased plasma lipid levels. Both, NRTI's and PI's regimens, have been shown to induce changes in mitochondrial DNA-polymerase γ .²⁶

1.4.3 Glucose Abnormalities and Insulin Resistance

Insulin resistance, glucose intolerance and Type II Diabetes Mellitus are included in the plurimetabolic syndrome associated with HAART. The mechanism by which glucose metabolism is adversely affected is poorly understood; however, it is suggested that PI's have an inhibitory effect on glucose transport. Furthermore, there is evidence to show that PI's harmfully affects beta-cell function.³⁸ Hyperglycaemia is consequential due to multiple factors: decreased glucose tolerance, increased peripheral insulin resistance, lower insulin clearance rate and increased fasting insulin. Increased cortisol levels also contribute to defective glucose metabolism.²⁵

A recent observational study in Costa Rica found the prevalence of insulin resistance in HIV-infected patients receiving HAART to be 34.2%. Interestingly, this figure is double the prevalence of insulin resistance in the general population in Costa Rica. Eighty eight percent of the HIV-infected patients were on an NRTI-based regimen.³⁹

1.4.4 Bone and other Endocrine Abnormalities

The prevalence of bone demineralisation is high in HIV-infected women. The contribution of HIV-infection and treatment regimens is uncertain. In an observational study, HAART was associated with osteopenia and osteoporosis. The results were more significant when PI's were used.⁴⁰ PI's are powerful inhibitors of the cytochrome P450. Three cytochrome P450 oxygenases are involved in controlling the serum 1,25 dihydroxyvitamin D₃ (1,25(OH)₂D₃), responsible for vitamin D₃ activities in bone tissue. An *in vitro* study illustrated that PI's suppress the bioactivation of 25- and 1 α -hydroxylase, which are vital in 1,25(OH)₂D₃ synthesis.⁴¹

The severity of disease influences metabolic parameters. In an Iranian observational study a lower CD₄ count (<250) was associated with low triglyceride and parathyroid hormone levels. Patients on an NRTI-regimen alone had lower levels of calcium but a regimen with an NRTI in combination with a PI or NNRTI was associated with an increased phosphorus level.²⁷

In view of the metabolic abnormalities associated with ART, the question arises what the effects on breastmilk composition could possibly be. A recent South African observational study showed no differences in the nutritive breastmilk composition (total energy, protein, carbohydrates, fat, calcium and phosphate) of HIV-infected women on ART compared to ART-naïve women.⁹ However, the effects of HIV and ART on the breastmilk composition of mothers giving birth to premature infants are largely unknown.

The following section describes the effects of HIV and ART on birth outcomes.

1.5 MATERNAL HUMAN IMMUNODEFICIENCY VIRUS INFECTION AND BIRTH OUTCOMES

The heavy burden of maternal HIV infection has contributed to the prevalence of adverse pregnancy outcomes.⁴² In developing countries, such as South Africa, maternal HIV infection may be associated with premature birth;^{5, 6} however, data from the developed world indicated no association between maternal HIV infection and premature birth.² Infants born to HIV-infected mothers were found to have direct adverse effects on organ systems such as the central nervous system, thymus and immune system that may affect growth,⁶ neurodevelopment, metabolic and other childhood aberrations.^{43, 44} These adverse effects correlate directly with the stage of HIV infection.⁵

Furthermore, the effects of HAART on birth outcomes are still indefinite. In HIV-infected mothers the role of HAART in the enhancement of maternal health and PMTCT of HIV is widely supported by the literature.⁴⁵⁻⁴⁷ When HAART is available, HIV transmission can be reduced substantially to 1- 2% in better-resourced settings,⁴⁸ ; however, it is largely uncertain what the effects of *in utero* exposure to HAART are on premature birth.⁴⁹ Combination ARV regimens (≥3 ARV drugs), are recommended for all pregnant women with CD₄ count ≥350cells/mm³ but the risk combination therapy for preterm birth is unclear.⁵⁰ A meta-analysis of 14 studies found no increased risk of premature births for women on ART

compared to women on no ART, but there was an increased risk for women on PI compared to non-PI regimens to deliver babies prematurely.^{50, 51} Later studies showed conflicting results concerning combination ART regimens, and specifically PI's, and premature birth.⁵⁰ A randomised control trial in Botswana showed a two-fold increase in the preterm birth rate for women who received lopinavir/ritonavir compared to those who received a triple nucleoside ART regimen.⁵² In contrast, in 2011 a retrospective cohort study that reviewed data from 3273 HIV-infected women from Malawi and Mozambique receiving triple ART showed that the use of ART reduced adverse pregnancy outcomes. The incidences of stillbirths/abortions and prematurity were significantly increased in women who did not receive ART compared to women on ART. The results also showed that LBW was not associated with ART use.⁷

African studies reported conflicting results with respect to maternal HIV-infection and LBW deliveries.⁵³⁻⁶² Intra-uterine growth restriction (IUGR), defined as a foetal weight, length or head circumference of <10th centile; may, among other factors, be responsible for premature delivery.¹ Premature birth and IUGR are major causes of paediatric morbidity and mortality.¹

IUGR can be symmetrical (weight, length and head circumference is small for gestational age [SGA]), indicating an abnormal process present since early in the pregnancy, or asymmetrical (sparing of the head circumference and length), indicating a pathology later in the pregnancy.⁶³ In an SA study among IUGR premature infants, there was a nonsignificant increased trend for HIV-infected mothers to give birth to a symmetrically growth-restricted infant (29%) compared to an asymmetrically growth-restricted infant (23%).⁶⁴ This possibly indicates the perinatal effects of maternal HIV status.⁶⁴ Studies conducted in the developed world showed no association between *in utero* exposure to HAART and IUGR,^{5,65,66} but results obtained from the developing world are conflicting.⁶⁶⁻⁶⁸

1.6 MATERNAL NUTRITIONAL STATUS AND BIRTH OUTCOMES

Maternal nutritional status during pregnancy is a key factor in determining foetal growth and anthropometrical parameters at birth. In South Africa poor household food security and the effects of HIV/AIDS Wasting Syndrome (or the combination thereof) may contribute to maternal undernutrition.⁶⁹ In the undernourished mother, the supply of maternal- foetal

nutrients are diminished thereby retarding foetal growth. Evidence suggests that chronic maternal undernutrition adversely affects foetal growth more than poor nutrition during pregnancy alone.⁷⁰ In an SA study, birth weight was inversely associated with maternal HIV status and mid-upper arm circumference (MUAC).⁴ Moreover, in multiparous pregnancies, fetuses compete for available nutrients ultimately resulting in IUGR.⁷¹

Foetal growth is susceptible to maternal nutritional deficiencies (specifically of protein and micronutrients) during the pre-implantation period as well as the period of rapid placental growth.⁷¹ Compromised synthesis of nitric oxide, an important vasodilator and angiogenesis factor, and polyamines (involved in protein synthesis) produces proof that maternal over- and undernutrition can both be responsible for IUGR.⁷¹ Furthermore, maternal nutritional status can be responsible for modifications in gene expression of the foetal genome through DNA methylation and histone adjustments.⁷¹ Changes in the intrauterine nutrition - and endocrine environment may have serious and permanent consequences for the infant through foetal programming. This hypothesis proposes that chronic diseases; vascular, endocrine or metabolic, that present later in life, may be a result of early foetal adaptations to malnourishment.^{72,73}

In addition to undernutrition, the incidence of overweight and obesity, especially in women, is rising dramatically in South Africa.⁶⁹ Overnutrition is associated with various health problems. Maternal overnutrition impedes placental growth and ultimately leads to growth faltering, significantly increasing neonatal morbidity and mortality.⁷²

In view of the vulnerability of the premature infant, breastmilk is regarded as an important feeding strategy for the premature infant.⁸ In the following section breastmilk and its nutritive components will be discussed to illustrate the importance of optimally feeding the HIV-exposed premature infant.

1.7 BREASTMILK AS A THERAPEUTIC STRATEGY FOR PREMATURE INFANTS

It is well documented that irrespective of HIV infection, ART regimen and maternal nutritional status, LBW premature infants are more vulnerable to complications and death than their term counterparts.^{4, 74} Premature infants have higher nutritional requirements during the neonatal period to facilitate growth compared to any other stage of life. This is due to several mechanisms including: i) LBW or IUGR resulting from a decreased time

exposed to intrauterine nutrition ii) medical complications related to prematurity, including hypoxia, hypotension, acidosis that ultimately increase nutritional requirements and iii) immature organ systems such as an immature gut, decreased enzymatic activity, reduced gastric motility and corticosteroid therapy. Therefore, it is vital to provide optimal nutritional therapeutic strategies to ensure better outcomes. Nutritional support corrects growth restriction and facilitates an appropriate weight gain tempo, which is nearly double that for a term neonate.⁷⁵ Feeding a premature infant breastmilk is the gold standard for feeding and has been proven to reduce morbidity and mortality. Human breastmilk is ideal for the neonate's immature organs that are not well adapted to responding to excesses or deficiencies of certain nutrients.⁷⁶

It is well documented that early nutrition has important effects on the short- and long-term outcomes in premature infants. The short- and long-term benefits of feeding a premature infant breastmilk are depicted in Table 1.2.

Table 1.2: Short- and long-term benefits of providing breastmilk to premature infants

Short-term benefits	Long-term benefits
<ul style="list-style-type: none"> ▪ Improved gastro-intestinal maturation ▪ Improved nutrient absorption ▪ Advanced cognitive development ▪ Improved eye development ▪ Improved host defence against infections ▪ Stronger mother-infant bonding ▪ Improved protection against Necrotizing Enterocolitis (NEC) 	<ul style="list-style-type: none"> ▪ Advanced mental development ▪ Improved motor development ▪ Higher intelligence scores ▪ Better visual function ▪ Healthier body composition in adolescence ▪ Lower incidence of metabolic syndrome in adult years

Adapted from: Picciano MF, et al.⁷⁶

1.8 THE NUTRITIONAL COMPOSITION OF PRETERM BREASTMILK

Table 1.3 depicts the nutritional composition of preterm breastmilk according to the number of days postpartum.

Table 1.3: Changes in preterm breastmilk composition in the neonatal period

Component	Preterm breastmilk per 100ml			
	Day 3	Day 7	Day 14	≥ Day 21
Energy (kCal)	49	67	70	67- 78
Carbohydrate (g)	6.2	6.9	7.0	7.3
Lactose (g)				6.7-7.3
Total nitrogen (g)	0.4	0.32	0.27	2.89
Total protein (g)	2.0	1.6	1.3	1.62-2.2
Casein (g)	0.6	0.48	0.4	0.49
Whey (g)	1.4	1.12	0.91	0.5
Total lipids (g/dl)	1.6	3.5	3.9	3.5-4.8
Water-soluble vitamins				
Ascorbic acid (mg)				4.4
Thiamine (µg)				8.9
Riboflavin (µg)				27
Niacin (mg)				210
Pyridoxine (mg)				6.2
Folate (µg)				3.1
Vitamin B ₁₂ (µg)				0.02
Pantothenic acid (µg)				230
Biotin (µg)				0.54

Fat-soluble vitamins				
Vitamin A (IU)				48
Vitamin K (µg)				2
Vitamin D (IU)			8	
Vitamin E (IU)				0.39
Major minerals				
Calcium (mg)				20-29.2
Magnesium (mg)				2.43-4.14
Phosphorus (mg)				9.3-15.5
Sodium (mg)				24-51
Potassium (mg)				42-70
Chloride (mg)				58
Trace minerals				
Iron (mg)		0.135		0.09
Zinc (mg)		0.37-0.62	0.98	0.37
Copper (mg)		0.506-0.57	0.489	0.038
Manganese (µg)		0.133	0.127	0.125-0.36
Selenium (µg)		11.8	11.4	2.4-11.4

Adapted from: Koletzko B,⁷⁸ Anderson DM et al,⁷⁹ Bishara R et al,⁸⁰ Bauer J, Gerss J,⁸¹ Kim SJ et al,⁸² Ronayne de Ferrer PA, et al,⁸³ Tsang RC et al.⁸⁴

1.8.1 Protein and amino acid composition of preterm breastmilk

The proteins in human breastmilk are hormone regulated and therefore the composition varies.⁸⁵ Breastmilk has two protein fractions defined by the solubility in acid: whey and casein. Preterm breastmilk has a whey protein predominant (whey:casein = 70:30) composition to facilitate rapid gastric emptying, enhance protein digestion and contains other whey-containing proteins resistant to hydrolysis (lysozyme, lactoferrin and immunoglobulin A involved in the enhancement of the host defence system).⁸⁶ Whey predominance also facilitates an optimal amino acid composition associated with enhanced brain function. Premature infants fed either mother's own milk; or a whey - or casein dominant formula were compared in a randomised controlled trial. Levels of methionine, phenylalanine and tyrosine were the lowest in the group that received breast milk. High levels of these amino acids impede brain development.⁸⁷ Of the 70% whey, alpha-lactalbumin, a component of the lactase synthetase complex, makes up 41% of the whey and 28% of the total protein content. The high proportion of alpha-lactalbumin is unique to human milk.⁸⁸ Alpha-lactalbumin is involved in mineral absorption (especially calcium and zinc), shows antibacterial and immune stimulatory actions and has an anti-infective property by enhancing apoptosis.⁸⁹

Conditional essential amino acids (particularly cysteine and taurine) can be synthesised by *de novo* synthesis in adults, but not by premature infants due to biochemical immaturity. Preterm breastmilk is rich in cysteine which is involved in some important metabolic functions such as growth and protein synthesis; and serves as the precursor of the potent antioxidant, glutathione.⁹⁰ Preterm breastmilk is also abundant in the amino acid taurine, which is imperative for fat absorption, improves the maturation of Auditory Brainstem Evoked Response (ABER)^a and is present in the osmoregulation of the central nervous system.⁹¹

^a Brainstem auditory evoked response (BAER) is a test to measure the brain wave activity that occurs in response to clicks or certain tones.

1.8.2 Carbohydrate composition of preterm breastmilk

Carbohydrates in preterm milk mainly consist of lactose and oligosaccharides (40% of the total energy). Lactose is well absorbed and is responsible for activating intestinal lactase. About 10% lactase is excreted in the stool for the functions of serving as a stool softer, the absorption of minerals and the beneficial composition of faecal bacteria.⁸⁶ Human milk oligosaccharides (HMOs) are studied frequently in the literature for their prebiotic effects and microorganismic anti-adhesive properties, preventing pathogenic attachment to the intestinal epithelial cells.⁹² In a recent SA study, Van Niekerk et al made an interesting discovery about HMO oligosaccharides that differed between HIV-infected and HIV-uninfected mothers who had given birth to premature infants. HIV-infected mothers had a higher HMO content in their breastmilk than HIV-uninfected mothers.¹⁰

1.8.3 Lipids and fatty acid composition of human preterm milk

Breastmilk comprises 98% triacylglycerols, 1% phospholipids and 0.5% cholesterol esters.⁹³ Lipids are emulsified in the aqueous phase of milk. Lipid globules are covered by bipolar materials that act as an emulsion stabilizer.⁹⁴ Half of the calories in preterm breastmilk are derived from lipids. Lipids act as an important energy source for the growing infant⁸¹ and the long-chain fatty acids in breastmilk has been shown to enhance brain and visual development in preterm infants.⁹⁵

Because of the immaturity of the preterm infant's digestive system, lipids in preterm breastmilk are adapted for optimal digestion. Due to the complex structure of the lipids and the presence of fatty acids and bile salt-stimulated lipase, the lipid composition in preterm breastmilk is of high suitability as these characteristics promote absorption. Medium-chain triglycerides account for less than 12% of the total fat in preterm milk, which also facilitates enhanced absorption.^{96,97}

1.8.4 Fatty acids in preterm breastmilk

Essential fatty acids are structural constituents of all tissues and are crucial for cell membrane synthesis of the brain, retina and neural tissue. Fatty acids also serve as precursors for eicosanoids that regulate many cell and organ functions.⁹⁵

The maternal diet largely determines the ratio of fatty acids in breastmilk.⁹⁸ Humans cannot synthesise essential fatty acids and therefore rely on the diet for the provision of essential

fatty acids, such as linoleic acid (LA), alpha-linolenic acid (ALA) and other long-chain polyunsaturated fatty acids (LC-PUFA) metabolites. During the last trimester of pregnancy and during the first year of life, docosahexanoic acid (DHA) is incorporated into the cells of the brain and retina.⁹⁹ The cerebral concentration of arachidonic acid (AA) and DHA increases rapidly during the growth spurt that occurs in the final trimester and the first few months thereafter.¹⁰⁰ Specifically DHA, the precursor of omega-3-polyunsaturated fatty acids (PUFAs), has been found to enhance cognitive function and improve visual acuity in term and preterm infants.^{101, 102} Throughout the final trimester of pregnancy the foetus requires 40- 60mg/kg omega-3-PUFA per day.¹⁰² In a regression analysis among a group of 73 children 6.5 years of age, a study found that 76% of intelligence quotient (IQ) variance could be attributable to the period of breastfeeding, week of gestation and the DHA and AA content in breastmilk.¹⁰²

An infant is incapable of producing DHA and is dependent on breastmilk to provide these fatty acids.¹⁰¹ Lucas and colleagues showed that premature infants fed mother's own milk (human breastmilk) had higher developmental scores at 18 months. The researchers later showed that these infants performed better in intellectual tests at 7.5 and 8 years of age. The advantage in IQ-score was breastfeeding dose dependent.¹⁰³ Additionally, the provision of breastmilk revealed advantages for visual development and psychomotor development at 18 months.¹⁰³ Retinopathy, a complication of prematurity, was less prevalent among premature infants fed breastmilk. Furthermore, breastmilk might relieve the degree of retinopathy. The improvement in visual function is related to the LC-PUFA and antioxidant content of breastmilk that improves the integrity and function of the retinal membranes.^{95,104}

Some studies have reported positive outcomes in visual function, long-term neurodevelopment and growth when omega-3 LC-PUFAs were supplemented in pregnancy and/or lactation. In a review by Campoy and colleagues, they warned to interpret the results with caution because the studies included might have methodological flaws.¹⁰⁵

LC-PUFAs are important for their part in modulating the immune system. In breastfed infants, LC-PUFAs, specifically DHA and AA, play an important role in T-cell development and

function.¹⁰⁶ Furthermore, there is evidence that LC-PUFAs exhibit virucidal capacity against encapsulated viruses.^{107, 108} A Tanzanian case-control study nested in a cohort study showed that increased concentrations of omega-6 PUFAs protected against MTCT of HIV.¹⁰⁷ Hsu proposed that HIV-infection destabilised the K⁺-channel, causing destruction. When DHA and AA are supplemented, the ability of the cell membrane to maintain its stability is restored.¹⁰⁹

1.8.5 Cholesterol composition of preterm breastmilk

Cholesterol, an integral part of human cell membranes, is found in abundance in breastmilk. The cholesterol quantity in breastmilk can fluctuate during the course of the day.¹¹⁰ Studies have shown that breastfed infants have lower blood cholesterol levels in adulthood.^{111, 112} It is hypothesised that exposure to high levels of cholesterol during lactation has a metabolic advantage on fatty acid synthesis that protects against hypercholesterolaemia later in life.¹¹³

1.8.6 Vitamin composition of preterm breastmilk

Premature infants' vitamin requirements are higher than term infants' due to higher utilisation and losses. There are currently insufficient data to know the exact amounts needed. Breastmilk provides insufficient quantities of vitamins to meet the high demands of preterm infants.¹¹⁴ Table 1.4 provides a summary of the vitamin composition of premature breastmilk.

Data on the breastmilk vitamin composition of HIV-infected women who gave birth to premature infants are scarce. However, it is known that HIV-infected patients frequently have vitamin deficiencies.¹¹⁵ It is recommended that lactating women take a multivitamin during the period of lactation. In the absence of ART, interventions that enhance immunity, such as vitamin supplementation during lactation, may decrease the risk of MTCT of HIV through breastmilk.¹¹⁶

Table 1.4: Summary of vitamin composition in breastmilk of women who gave birth to premature infants

Vitamin	Description
Vitamin A	<ul style="list-style-type: none"> ▪ The vitamin A content of premature breastmilk is dependent on dietary intake.^{78, 85} The average vitamin A content of breastmilk is 75 mcg/dL (280IU/dL) and some of it is present in the form of β-carotene. ▪ In a Kenyan randomised control trial, severe vitamin A deficiency (<20 mcg/dL) was associated with HIV-infection, thus increasing the risk of MTCT.¹¹⁷ ▪ However, vitamin A supplementation was associated with an increased risk of MTCT of HIV through breastmilk.¹¹⁶
Vitamin D	<ul style="list-style-type: none"> ▪ Vitamin D is present in the aqueous and lipid fractions of human breastmilk. The form in which vitamin D is available is 25-OH₂ vitamin D. Infants require sunlight exposure to convert 25-OH₂ vitamin D to the – active form 1,25-(OH)₂ vitamin D. A ten-fold increase in the vitamin D content of breastmilk is possible when the mother obtains exposure to sunlight.^{85, 118} ▪ Vitamin D deficiency is prevalent (55.6%) in HIV-infected patients¹¹⁹ and is associated with accelerated disease progression.¹²⁰

Vitamin E	<ul style="list-style-type: none"> Colostrum has a high concentration of vitamin E (600 mg/l), which decreases by a third in mature preterm breastmilk.⁷⁸ The average vitamin E (tocopherol) content of breastmilk is 40 mcg/g of lipid.^{85,121} The vitamin E content is adequate to fulfil the neonate's requirements and is present in the beta- and gamma-tocopherol in mature milk.⁸⁵ The amount of vitamin E increases during the course of a breastfeed, and hindmilk has four times the amount of vitamin E than foremilk.⁷⁸
Vitamin K	<ul style="list-style-type: none"> Vitamin K is scarce in breastmilk (2.3mcg/dL) and infants require a postnatal vitamin K injection (0.5-1.0 mg) to avoid a deficiency.⁸⁵
Vitamin B	<ul style="list-style-type: none"> Unlike the other water-soluble vitamins, vitamin B₁₂ and folic acid are whey protein-bound and are not influenced by the maternal diet. Known factors to influence the composition are gestational age and hormones.⁸⁵ In a randomised control trial lower baseline vitamin B₁₂ levels correlated with lower CD₄ count.¹¹⁵
Vitamin C	<ul style="list-style-type: none"> Dietary intake and not supplemental intake strongly affects the vitamin C concentration in breastmilk.¹²²

1.8.7 Mineral composition of breastmilk

Minerals in breastmilk are central in the optimal infantile growth. Breastmilk has a lower mineral content compared to other mammalian milk to reduce the strain on the kidneys and because humans show a slower growth velocity to other mammals. The osmolarity of breastmilk is largely dependent on the mineral content.⁸⁵ Key minerals in breastmilk include sodium (Na), phosphorus (P), calcium (Ca), magnesium (Mg), and chloride (Cl).¹²³ Most of the minerals decrease over a few months of breastfeeding, with the exception of magnesium that increases.¹²³ Studies showed that the net magnesium retention from preterm breastmilk is equal to the intrauterine requirements.^{125, 126} The bioavailability of minerals, such as calcium, magnesium, iron and zinc, is excellent and this not only improves the absorption of these minerals, but also reduces the burden on the lactating mother to produce large quantities of these elements.^{85, 127}

The minerals selected for discussion in this section have relevance to section 2.11.2.3 in the methodology. These minerals relate to the growth and development of premature infants.

i. Phosphorus (P)

One of the major roles of phosphorus for premature infants is for ensuring dental-¹²⁸ respiratory-¹²⁹ and bone health.¹³⁰

One study found that among 76 very-low-birth-weight (VLBW) infants, 62% had enamel defects associated with hypophosphataemia. The results indicated that the only significant variable in the logistic regression analysis was that infants with a 1-mg/dL increase in serum phosphorus levels had a 68% reduction in the odds of having enamel hypoplasia.¹²⁸

Premature infants are at substantial risk to develop respiratory distress syndrome and chronic lung disease. Phosphorus, among other nutrients, was identified to be significant in lung development and function.¹²⁹

Premature infants are at a high risk of developing osteopenia of prematurity due to multiple reasons: i) Reduced stores due to a shorter gestation, ii) Inadequate supply of phosphorus (and other nutrients) due to problems in establishing full enteral feeding/ breastfeeding, iii) low concentration of phosphorus in breastmilk iv) vitamin D deficiency and v) missing a period of mineral accretion in the last trimester of pregnancy.¹³⁰ In order to achieve bone

mineralization, premature infants require an enteral supply of 60- 90mg/kg/day of phosphorus salts.¹³¹ Given the low concentration of phosphorus in breastmilk, it is impossible to achieve the requirements without fortification. It is considered standard practice to fortify breastmilk of mothers of premature infants.¹³² The breastmilk fortifier available in South Africa (FM85) provides 900mg P/100g which is sufficient to meet phosphorus needs of premature infants on full feeds.¹³³

ii. Iron (Fe)

Although the iron concentration in breastmilk is low compared to other mammals, it is highly bioavailable. This can be illustrated by the fact that only 10% of iron in cow's milk is available for absorption, compared to almost half (49%) of breastmilk that is bioavailable.^{85, 134}

Premature infants experience a sudden reduction of erythropoiesis at birth, which leads to anaemia of prematurity. Additionally, premature infants are born with low iron stores and often lose blood during phlebotomy.¹³⁵ It is recommended that preterm infants receiving breastmilk be supplemented with 2- 4mg/kg/day iron starting on day 14 of life.¹³⁶ A systematic review showed that preterm infants who received iron supplementation, had higher haemoglobin levels and -iron stores and were less likely to have iron deficiency anaemia.¹³⁷

iii. Zinc (Zn)

Many studies assessed the zinc concentration of breastmilk, largely because of its role in growth,^{83, 136} cell differentiation and macronutrient metabolism.¹³⁹ Growth is therefore the major factor in determining zinc requirements.¹³⁹ A preterm infant needs to retain 25% of the zinc to master *in utero* accumulation.¹⁴⁰ The zinc bioavailability in preterm breastmilk is higher than other in mammalian milk due to the strong binding of zinc to casein. This is demonstrated by the fact that 60% of zinc in preterm breastmilk is absorbed compared to 14% in preterm formula.¹³⁹ The zinc concentration in human breastmilk is not dependent on maternal diet. The breastmilk zinc concentration in preterm milk is significantly higher than term breastmilk and zinc concentration is highest in transitional milk and then decrease over a period of breastfeeding.¹³⁹

The results of a Cochrane review by Siegfried and colleagues indicated that there are currently no indications to supplement HIV-infected pregnant or breastfeeding women with zinc. They suggested that specific requirements for HIV-infected breastfeeding women should be developed and that more research should be done on micronutrient supplementation during the different stages of HIV.¹⁴¹

iv. Copper (Cu):

The copper content in preterm breastmilk is adequate to fulfil the premature infant's requirements. Copper is vital in anti-oxidant defences and forms part of superoxide dismutase that protects cell membranes against reactive oxygen species. Copper is stored in the liver and transported to peripheral tissues bound to ceruloplasmin whose production starts six to 12 weeks postnatally. However, copper deficiency is rare in neonates. Copper deficiency presents as a hypochromic anaemia not responsive to iron supplementation.¹³⁹

v. Other minerals: Selenium (Se)

Selenium is a potent anti-oxidant and plays an important role in cell-mediated immunity and in brain, thyroid and cardiovascular health. HIV-infected people are more likely to have a selenium deficiency, because the body uses selenoproteins to suppress viral replication or the virus uses selenium to generate its own selenoproteins. In a Malawian randomised control trial, a sodium selenite dietary supplement did not significantly affect HIV-infected breastfeeding women's breastmilk selenium content. However, the selenium in the dietary supplement is not as well absorbed as in many selenium-containing food sources.¹⁴² In another African trial, selenium supplementation increased HIV-RNA in breastmilk among primiparous HIV-naïve women.¹⁴³

Preterm breastmilk composition is highly variable. Numerous factors affect preterm breastmilk composition between individuals. These include: differences in foremilk and hindmilk, stage of lactation, prematurity, maternal dietary intake, maternal nutritional status, breastmilk expression technique and pasteurisation methods used. The following section will discuss each factor in detail.

1.9 FACTORS THAT INFLUENCE THE NUTRITIVE COMPOSITION OF HUMAN BREASTMILK

1.9.1 Foremilk and hindmilk

It is well known that the composition of the within-feed nutrient content of breastmilk changes to accommodate the infant's needs. The water content between the the foremilk and hindmilk does not change significantly. Because the lipid content increases linearly with the duration of the feed, the energy content of the hindmilk is higher than those of the foremilk.⁷¹ Lipid amounts in hindmilk are double that in foremilk. Protein and lactose decrease during the course of the feed, but this is inversely proportionate to the rise in lipid content.^{144,145}

For relevance of data collection procedures, breastmilk samples from the left and right breast do not differ significantly with regards to major nutrients (sodium, potassium, urea, creatinine, phosphate, calcium, magnesium, protein, carbohydrate and lipids) that contribute to more than 90% of the osmotic activity of human breastmilk. The fat content of the hindmilk can increase two- to three-fold,¹⁴⁴ and phospholipids and cholesterol decrease between the foremilk and hindmilk.¹⁴⁴ Although the total fat content increases, the fatty acid content of foremilk and hindmilk does not differ significantly.¹⁴⁶ These findings have an impact on the sampling of breastmilk. If the water content of the milk is of interest, the sample can be taken at any stage of the feed. However, if the lipid content is of interest, the mid-feed sample will give a representative sample. Between-breast variation does sometimes occur and thus it is recommended to obtain a sample from each breast. If the sodium and potassium content is above the normal values, the possibility of mastitis should be investigated.¹⁴⁴

Interestingly, in a Nigerian study, VLBW preterm infants were randomised to receiving either hindmilk or composite breastmilk for two weeks. The results indicated that the first group had significantly better weight gain rates.¹⁴⁷

1.9.2 Stage of lactation

The nutritional composition of preterm breastmilk changes according to postpartum phase:¹⁴⁸

- i. Colostrum is the first secretion of the mammary glands at the time of parturition with the goal of providing passive immunity. Breastmilk produced in the first week after giving birth is high in colostrum..
- ii. Transitional milk occurs after colostrum and lasts for one to two weeks.
- iii. Mature milk is the final milk produced and is comprised of a high percentage of water.

Table 1.5 depicts the nutritive composition between colostrum, transitional and mature preterm breastmilk.

Table 1.5: Nutritive composition (per 100ml) of colostrum, transitional- and mature preterm breastmilk

Weeks postpartum	Energy (kCal)	Protein (g)	Fat (g)	Calcium (mg)	Phosphorus (mg)
Week 1 (Colostrum)	60 (45- 75)	2.2 (.03- 4.1)	2.6 (0.5- 2.7)	26 (9- 43)	11 (1- 22)
Week 2 (Early Transitional Milk)	71 (49- 94)	1.5 (0.8- 2.3)	3.5 (1.2- 5.7)	25 (11- 39)	15 (8- 21)
Week 3-4 (Late Transitional Milk)	77 (61- 92)	1.4 (0.6- 2.2)	3.5 (1.6- 5.5)	25 (13- 36)	14 (8- 20)
Week 10-12 (Mature Milk)	66 (39- 94)	1.0 (0.6- 1.4)	3.7 (0.8- 6.5)	29 (19- 38)	12 (8- 15)

Adapted from: Gidrewicz, Fenton.¹⁴⁸

As demonstrated in Table 1.5 the total energy, lipids and minerals increase within the first month of lactation while the protein content decreases. Similarly, lactose concentration in preterm breastmilk has been shown to increase in the first month of lactation.¹⁴⁹

A study that evaluated the changes in fatty acids in preterm breastmilk over 26 weeks of lactation showed the following results: The monounsaturated and saturated fatty acids remained relatively stable in the six-month period. Linoleic acid and alpha-linolenic acid showed an increasing trend from transitional to mature milk (8.7% to 9.9% of the total fatty acids). The LC-PUFAs were highest in Week 1 and then showed a decreasing trend.¹⁵⁰

1.9.3 Prematurity

The degree of prematurity affects the composition of preterm breastmilk. The nutrient density of preterm breastmilk shows an inverse relationship with gestational age.¹⁵¹ The energy content of mature preterm breastmilk varies between 65 and 78kCal/100ml.⁷¹ Table 1.6 depicts the elevated nutrient composition of preterm breastmilk to accommodate growth.

Table 1.6: Nitrogen, fatty acid and energy content of preterm and term breastmilk

Gestational age	Nitrogen (mg/dl)	Fatty acids (g/dl)	Energy (kCal/dl)
26-31 weeks	297	4.46	78.6
32-36 weeks	250	3.94	69.0
Term	259	3.20	66.6

1.9.3.1 Protein composition

The protein composition of preterm breastmilk is described in Section 1.9.1 of this chapter. The protein content of breastmilk is relative to the infant's age.⁸¹ In a systematic review by Gidrewicz and Fenton, the true protein content of preterm breastmilk was found to be higher by up to 35% (0.7 g/dL) in the first three days after birth compared to term breastmilk. After that, the difference decreased by 0.2 g/dL and by weeks 10– 12, the difference disappeared.¹⁴⁹ Uncertainty exists as to which proteins contribute most to the elevated protein content of preterm breastmilk. The degree of difference in composition is related to gestational age and level of prematurity.⁸¹

1.9.3.2 Lipid composition

Refer to Section 1.8.3 for the complete analysis of lipid composition. The total lipids in preterm and term breastmilk did not show a significant difference in the systematic review by Gidrewicz and Fenton. However, there was a non-significant elevation in preterm breastmilk of 23% in the first three days of lactation.¹⁴⁸

In a systematic review of five studies comparing the fatty acid content of term and preterm breastmilk, no significant differences were observed for saturated and mono-unsaturated fatty acids between term and preterm breastmilk. However, the DHA content of preterm breastmilk were significantly higher than in term breastmilk. The mechanism of action is related to the maternal LC-PUFA stores. In the case of premature infants, the gestational period in which the maternal LC-PUFA were transmitted to the infant was interrupted. This resulted in a positive maternal LC-PUFA balance. In addition, a premature infant can only consume a limited breastmilk volume and therefore the LC-PUFA concentration of premature breastmilk is higher.¹⁵³

1.9.3.3 Carbohydrate composition

The carbohydrate composition of premature breastmilk is discussed in Section 1.8.2. Gidrewicz and Fenton found that the lactose concentration was significantly lower in preterm breastmilk than in term breastmilk with the exception of a few days where it was higher. Although there were limited data on the oligosaccharide content of preterm breastmilk, the researchers found it to be similar in preterm and term breastmilk.¹⁴⁸

1.9.3.4 Mineral composition

A complete discussion of mineral composition is given in Section 1.8.7. The mineral and electrolyte content of preterm breastmilk is significantly elevated when compared to term breastmilk. This is not only to accommodate the increased requirements for growth, but also to compensate for the high losses, especially of sodium.⁷⁹ However, Gidrewicz and Fenton found no differences in the calcium and phosphate concentration between preterm and term breastmilk.¹³⁷ In an observational study, Ejezie et al. found preterm breastmilk to be significantly higher in iron compared to term breastmilk.¹⁵⁴

1.9.4 The effect of maternal dietary intake on breastmilk composition

Various studies assessed the relationship between maternal diet and the breastmilk composition under diverse environmental circumstances. A lactating woman can provide adequate nutrients even when her diet is deficient in nutrients unless energy deprivation is extreme.¹⁵⁵

The maternal diet does not characteristically affect the quality or quantity of breastmilk protein, even in malnourished women. However, protein supplementation increases milk protein concentration, and free amino acid and urea content parallels the quantity and quality of maternal dietary protein. A study found that breastmilk nitrogen content was higher in women fed a high protein diet (134g protein per day) compared to women fed a low protein diet (46g protein per day).¹⁵⁶

Maternal diet affects the lipid profile and more importantly, the essential fatty acid profile of breastmilk; however, the total fat content is not affected by the maternal diet.^{81, 101} Observational and cohort studies have linked maternal fish intake during pregnancy and during lactation to the essential fatty acid content of breastmilk and consequently developmental outcomes and IQ. Maternal fish intake did not have a significant effect on infant blood mercury levels.¹⁵⁷⁻¹⁵⁹

The quantity of water- and fat-soluble vitamins, with the exception of vitamin K, is dependent on the maternal diet.⁸¹ Deficiencies of water-soluble vitamins are very rare, even when the maternal diet and breastmilk contain inadequate amounts.¹⁶⁰ Although the vitamin B₁₂ content of breastmilk is less determined by dietary intake, low levels have been reported in vegan mothers, mothers with pernicious anaemia, undernourished mothers and mothers with a poor intake of animal products.¹⁶¹⁻¹⁶³ Studies have reported vitamin B₆ to be the most likely water-soluble vitamin to be deficient in breastmilk.^{81, 163} One study found that the maternal diet had no effect on the breastmilk concentration of vitamin B₆.¹⁶⁴ Overall, the mineral content of breastmilk does not reflect the maternal serum mineral status.¹²⁴

1.9.5 The effect of maternal nutritional status on breastmilk composition

Pregnancy and lactation are a nutritionally demanding period and can lead to nutritional depletion, especially when lactation and a subsequent pregnancy overlap.^{165,166} The human body is uniquely adapted to adjust to the demands of lactation, provided that a well-balanced diet is consumed.¹⁶⁵ The International Dietary Energy Consultancy Group's 1993 Annual Report to the Administrative Committee on Coordination/Subcommittee on Nutrition concluded that nutritional status did not reflect the lactation capacity of a woman, except in the case of severe undernutrition ('famine or near famine conditions').¹⁶⁷

In theory, the lactating woman has higher nutritional demands to produce adequate nutrition for her infant.¹⁶⁸ For many women, this is not possible due to social reasons or a lack of food security. Women from the developed world have an approximate daily energy intake of 2600kCal compared to women of the developing world who consume only 1600kCal per day.¹⁶⁹ Lactating mothers from the developing world often do not meet the increased energy requirements during lactation. A diet chronic deficient in energy can lead to depletion which ultimately adversely affects breastmilk composition.¹⁵⁵ However, Prentice et al. illustrated that milk volumes from undernourished women in developing countries did not differ from well-nourished women from developed countries.¹⁷⁰ In this section the many different factors that influence the nutritive composition of human breastmilk were discussed.

The following sections (breastmilk expression techniques and pasteurisation) are of relevance for data collection procedures described in Section 2.12.4 in the methodology.

1.9.6 Breastmilk expression technique

Breastmilk expression is done manually or with a breastmilk expression pump.¹⁷¹ In South Africa the manual expression method is mainly used as it is cost effective and safe.¹⁷² The usage of a breast pump is in line with the WHO/UNICEF Code of Marketing of Breastmilk Substitutes¹⁷³ as long as feeding bottles do not accompany the pumps and are not inappropriately marketed.

The Food and Drug Administration (FDA) recommends FDA approved pumps for the use by one mother exclusively, because use by more than one mother increase the risk of HIV-1 transmission.¹⁷⁴

Manual expression produces a reduced milk volume and is more labour intensive compared to breastpump expression. A benefit of manual expression is that there is very little equipment to clean and sterilise in comparison with breast pumps.¹⁷¹ Breast pumps are very efficient and may pose significant advantages to mothers of preterm infants as they often struggle with low breastmilk volumes.¹⁷⁵

Using a pump is a robust way to ascertain the maintenance of breastmilk production for a longer period.¹⁷⁶ There is little evidence that compares manual and pump expression techniques; however, some studies have shown differences in protein, fat, sodium and potassium content.¹⁷⁷

1.9.7 Pasteurisation of breastmilk

Van Wyk et al. described the effect of the Pretoria pasteurisation method on the breastmilk composition of 32 Xhosa speaking South African mothers with VLBW premature (<34 weeks gestation) infants. Each milk sample was halved- one half was kept raw and the other was pasteurised. The results indicated no statistical difference in the breastmilk composition pre- and post-pasteurisation.¹⁷⁸

Another study compared the effects of Pretoria pasteurisation^b and Flash-heat pasteurisation^c on the nutritional properties in breastmilk. Both methods caused minimal nutritive losses on vitamins A, -B₆, -B₁₂, -C, -folate, -riboflavin and -thiamine. However, the Flash-heat Pasteurisation method was more effective in inactivating HIV. The WHO recommends the Flash heat pasteurisation method to prevent MTCT of HIV.¹⁷⁹

Concerns in pasteurising breastmilk were that preterm infants showed a decreased growth velocity, because the pasteurisation process destroyed the heat-labile lipase and that the

^b This method uses the principle of heat transfer from 450 ml water heated to boiling point in an aluminium pot to a smaller volume of breastmilk in a glass jar inserted in the water. Milk is heated to a temperature of 52-62°C for 10-15 minutes. Thereafter, milk is cooled to room temperature/37°C.

^c In this method expressed breastmilk in a glass jar is placed in 450 ml water in an aluminium pot over a heat source. Water is brought to boiling point/100°C (milk reaches an approximate temperature of 72°C) and thereafter cooled down to room temperature/37°C.

composition of donor breastmilk, used for pasteurisation, differs significantly that of preterm milk.¹⁷⁶ Pasteurisation not only destroys the harmful pathogens in breastmilk, but also diminishes the bacteriostatic effect which can result in pathogenic contamination.¹⁸⁰

1.9.8 Effect of HIV-infection status on human breastmilk composition

The results obtained by Van Wyk et al indicated that the breastmilk of HIV-1 positive mothers was as healthful as the breastmilk of HIV-1 negative mothers concerning the nutritive composition alone.¹⁷⁸ Another SA study found that HIV-infected women's breastmilk had elevated protein levels and decreased calcium levels compared to breastmilk of HIV-uninfected women who gave birth to term infants. There was no significant difference in breastmilk composition of HIV-infected women receiving ART compared to HIV-infected women not receiving ART (HIV-naïve). However, there was a significant correlation between the viral load and percentage total solids^d in the HIV-naïve women. A significant correlation also existed between the HIV-naïve women's CD₄ count and total breastmilk energy content. Interestingly, there was no correlation between the protein-, carbohydrate-, -fat-, calcium- and -phosphate levels and viral load showing that the difference could be in other untested components making up the nutritive composition of breastmilk.⁹

^d Total solids are the sum of the protein, carbohydrates, fat and ash/minerals in breastmilk.

MOTIVATION FOR THE INVESTIGATION

Premature delivery is a major global perinatal health problem with a rising incidence in Southern Africa.³ African HIV-infected women are more likely to give birth to premature infants.³⁻⁶ The SA HIV epidemic is regarded as a national health crisis that affects a great proportion of the population.¹¹ Statistics show that a third of SA women who attend antenatal clinics are HIV infected.¹²

Sufficient evidence is available to show that premature infants significantly benefit from human breastmilk in the short and long term in a dose-dependent way.⁷⁶ The American Academy of Pediatrics recommends human milk as the preferred feed for all newborns, including premature infants.¹⁸¹ Recent PMTCT policy changes¹⁴ as well as advancements in maternal and neonatal ART regimens proved that the provision of expressed breastmilk and/or breastfeeding is safe for the HIV-exposed premature infant.^{18,19} The side effects of ART include adverse effects on metabolic and endocrine systems, such as morphological body changes, dyslipidaemia, glucose intolerance and bone mineral abnormalities.²⁵

Breastmilk composition is highly variable among individuals.⁸⁶ It is well known that stage of lactation,¹⁴⁸ foremilk versus hindmilk,^{71,144} prematurity,⁸¹⁻¹⁴⁹ maternal diet¹⁴⁴, and pasteurisation method¹⁷⁹ influence breastmilk composition. Maternal nutritional status and breastmilk expression technique¹⁶⁷ do not influence breastmilk composition. Recently, SA data showed that there were differences in the breastmilk composition of HIV-infected and HIV-uninfected women who had given birth to term⁹ and preterm infants.¹⁰ To date, the effects of ART on breastmilk composition are unknown. The aim of this investigation was to evaluate the differences in breastmilk composition of HIV-infected women receiving ART and HIV-uninfected women. The link between maternal HIV infection and LBW and IUGR is controversial in African studies.⁵³⁻⁶² The effects of ART on birth outcomes are still indefinite.⁴⁹⁻⁵¹ The objectives of this investigation were to assess the anthropometric parameters of HIV-infected and HIV-uninfected mothers as well as to assess the neonatal anthropometric parameters of premature infants in relation to maternal anthropometric parameters, HIV status and ART regimen.

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CHAPTER 2:

Methodology

METHODOLOGY

In this chapter, the research methodology will be described. The research question, aim and objectives as well as the complete study plan are detailed in the following sections.

Research Question

Is there a difference in the breastmilk composition of HIV-infected mothers [receiving antiretroviral therapy (ART)] and HIV-uninfected mothers who gave birth to premature infants?

2.1 Aim of the investigation

The aim of the investigation was to assess and compare the breastmilk composition of HIV-infected (receiving ART) and uninfected mothers who give birth to premature infants.

2.2 Objectives of the investigation

The objectives included the following:

- i. To describe the HIV-infected mother population with reference to the use of ART treatment schedule and disease state
- ii. To compare the breastmilk components of HIV-infected mothers receiving ART, according to the ART regime with that of HIV-uninfected mothers
- iii. To assess the anthropometric parameters (weight, height and MUAC) of HIV-infected and -uninfected mothers
- iv. To assess the neonatal anthropometric parameters of the premature infants in relation to maternal nutritional status, HIV status and ART regime

2.3 Null Hypothesis

H_0 : There is no difference between the nutritive breastmilk composition of HIV-infected mothers receiving ART and -uninfected mothers who gave birth to premature infants.

H_0 : Maternal nutritional status, HIV status and ART regime do not affect neonatal nutritional status at birth

Figure 2.1 depicts a conceptual framework to address the aim and objectives of the investigation.

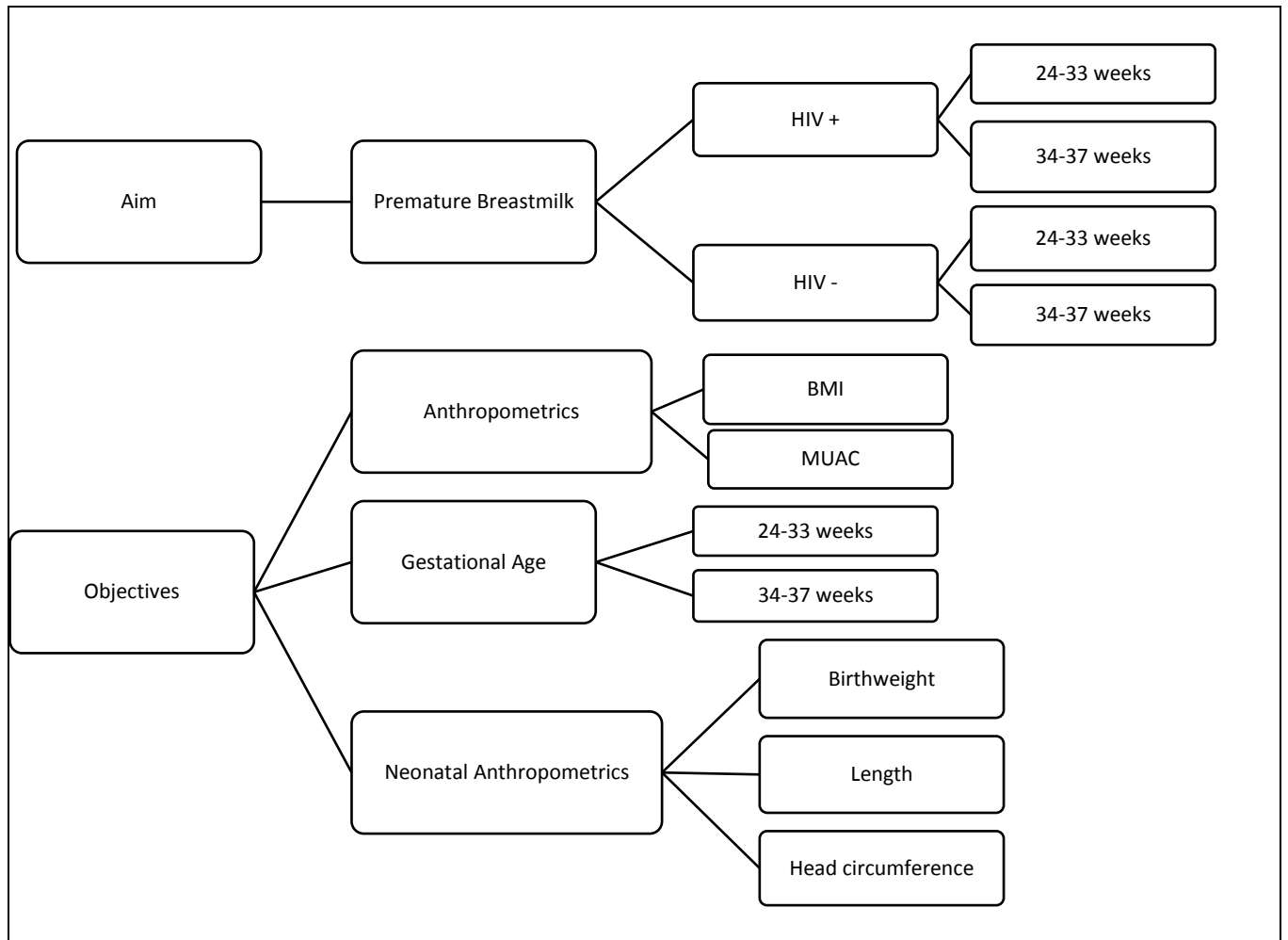


Figure 2.1: Conceptual framework to address the study aim and objectives

2.4 Study Design

The research entailed a cross-sectional descriptive study with an analytical component.

2.5 Setting

The study was conducted in the postnatal paediatric ward(s) of Kalafong Hospital, Pretoria. The wards included in the study were the Neonatal High Care and Intensive care unit (ICU) and the Kangaroo Mother Care (KMC) Unit.

2.6 Study population

The study population consisted of HIV-infected and –uninfected mothers who gave birth to premature infants. The infants of the mothers were also included in the study.

2.7 Sample size

The sample size was calculated by a 1-way ANOVA sample size calculation. In order to obtain an effect size (RMSEE) = 0.55, the sample size had to include 68 mothers (N=68). The four subgroups each had to include 17 mothers (n=17). Thus, the final sample had to include 17 HIV-infected mothers receiving ART who gave birth to preterm neonates 24-33 weeks gestation; 17 HIV-infected mothers receiving ART who gave birth to preterm neonates 34-37 weeks gestation, 17 HIV-uninfected mothers who gave birth to preterm neonates 24-33 weeks gestation and 17 HIV-uninfected mothers who gave birth to preterm neonates 34-37 weeks gestation; (p=0.05). Please refer to schematic diagram Figure 2.2 for a visual presentation of the information described here. Table 2.1 depicts the study setting with associated statistics (2013).

Table 2.1: Statistics on breastfeeding mothers at Kalafong Hospital

Ward	Number of breastfeeding mothers per day
Observation	10
High Care	16-20
ICU	6
TOTAL	32-36

The final sample consisted of 74 mother-infant pairs. This included 38 HIV-infected mothers and –exposed infants receiving ART; and 36 HIV-uninfected mothers and –unexposed infants. A total of 92 pairs were screened for inclusion in the investigation. Seventeen pairs did not meet the inclusion criteria of which twelve mothers were discharged before data collection procedures were completed, two mothers did not have adequate breastmilk volumes to provide a second sample, one mother were not on ART at the time of data collection and two mothers were excluded due to an ELISA test not performed. One mother declined consent (no questionnaire was filled in). Mothers whose breastmilk production were inadequate to provide the full sample over one or two days (methods discussed in section 2.11.2.3) were excluded due to ethical reasons. Figure 2.2 provides a schematic presentation of the sample. A total of 260 infants were admitted during the period of data collection.

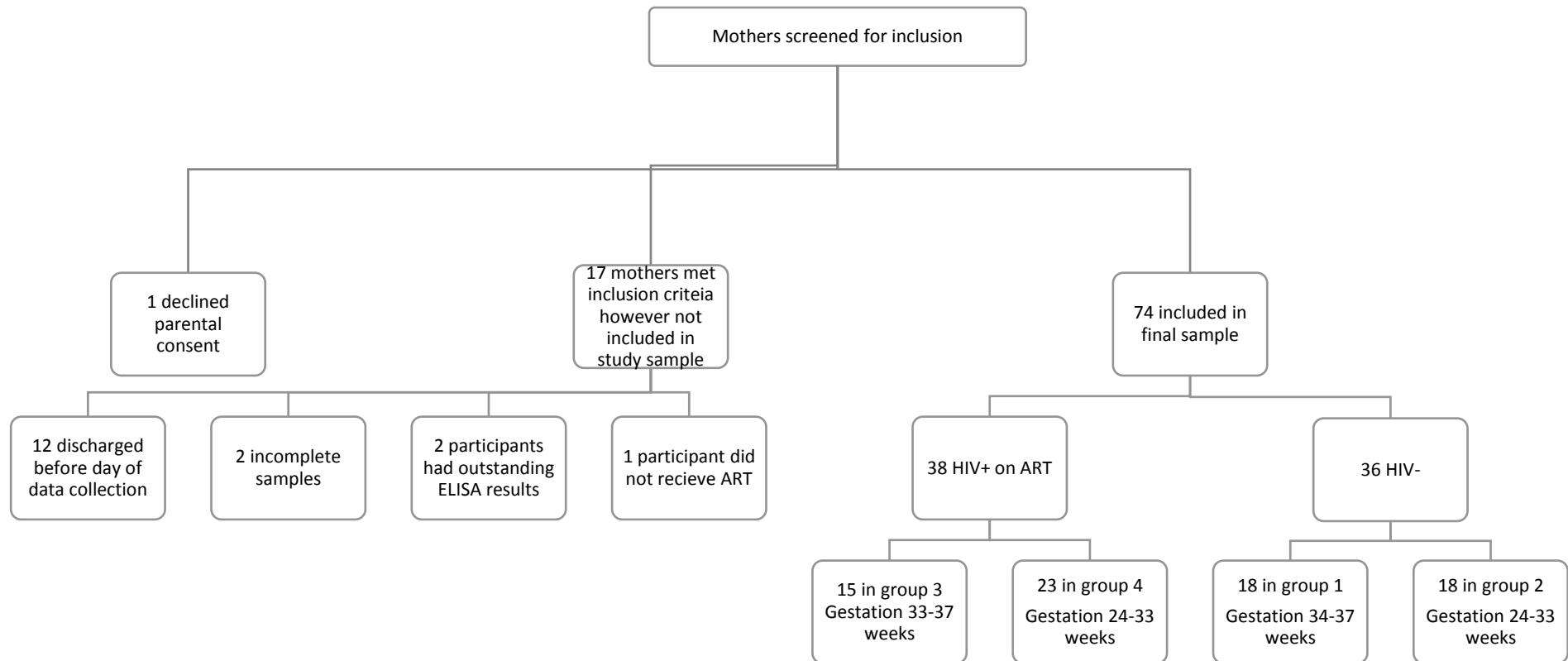


Figure 2.2: Flow diagram of mother-infant pairs included in the investigation

2.8 Sample Selection

All mothers and infants pairs who fitted the inclusion criteria and provided written informed consent were included in the study.

2.9 Inclusion Criteria

The inclusion criteria were as follows:

- I. All mothers who gave birth to live, premature infants (<37 weeks gestation) and who were willing to participate and provide written informed consent
- II. Only mothers who decided to breastfeed after counselling, regardless of their HIV status, were included. (All HIV-infected mothers are counselled routinely at Kalafong Hospital about the risks and benefits of breastfeeding and bottle-feeding relating to the prevention of mother to child transmission (PMTCT) programme)
- III. HIV-infected mothers who were on the (PMTCT) treatment regimen and if antiretroviral (ARV) medication was prescribed
- IV. Premature infants 24-33 and 34-37 weeks gestation
- V. First born infant of twins

2.10 Exclusion Criteria

All lactating mothers:

- I. Who gave birth to premature infants <500g or <24 weeks gestational age
- II. Who was breastfeeding infants/children from a previous pregnancy
- III. Whose infants did not have a completed Road to Health Booklet available or who's medical information were incomplete or unavailable
- IV. Who were unable to provide adequate quantities of breastmilk after feeding her infant(s)
- V. Who were known for substance abuse
- VI. With active Mycobacterium tuberculosis who were still potentially infectious
- VII. Who were receiving medication that are contra-indicated during lactation (Table 2.2)

Table 2.2: Medication contra-indicated during lactation¹

Classification	Drugs
Psychiatric therapy	Antipsychotics (chlorpromazine, lithium), Antidepressants (tricyclic agents)
Corticosteroids and Immune-suppressive therapy	Cyclophosphamide, Doxorubicin, Methotrexate, Gold salts, Propylthiouracil, Methimazole *Cyclosporine is safe for lactation
Antineoplastic therapy	Cisplatin, cyclophosphamide, methotrexate, doxorubicin
Anticonvulsants	Phenobarbital, ethosuximide, primidone
Drugs of abuse	Ethanol, amphetamines, opioids, phencyclidine
Ergot-alkaloids	Ergonovine, bromocriptine
Other	Radiopharmaceuticals, chloramphenicol, phenylbutazone, atropine, thiouracil, iodides, mercurals, warfarin, cytotoxics, amiodarone, estrogen-containing contraceptives, diuretics

2.11 Data Collection

2.11.1 Subject recruitment

Consecutive mother-infant pairs were included in the study. This included 38 HIV-infected mothers and –exposed infants receiving ART; and 36 HIV-uninfected mothers and – unexposed infants. The subjects were identified by the medical staff and verified by the most senior clinician. Staff (paediatricians, doctors and nurses) notified the primary researcher of admissions and possible candidates. On weekdays, the primary researcher attended the facility in the afternoons on a daily basis.

2.11.2 Maternal Information

2.11.2.1 Establishing HIV status

An HIV-infected status was established by a confirmed positive rapid HIV test during the prenatal period and an -HIV-uninfected status was confirmed with an ELISA test in the postnatal period.

The researcher ensured that all mothers provided accurate information regarding ART regimes and dosages by checking prescription charts (from hospital file and/or antenatal file) or asked the mothers to show her their home medication.

2.11.2.2 Questionnaire

A researcher-administrated demographic questionnaire, tested for face and content validity, was completed. A pilot study was conducted to ensure face validity of the questionnaire. The questionnaire was also reviewed by two experts in the field of paediatrics and HIV. All staff involved in the data collection was trained to standardise methods. Refer to Addendum A.

2.11.2.3 Breast milk sample collection

Samples of HIV-infected and HIV-uninfected breast milk were analysed. All breast milk samples were raw untreated specimens; therefore milk samples did not undergo any pasteurization procedures. Lactation consultants/ milk handlers^e were responsible for sample collection, coding and freezing of samples. Samples were frozen immediately upon collection. The researcher communicated information regarding participants and dates of sample collection to the lactation consultants.

Breast milk was collected in sterilised containers with lids. Expression of breast milk was done by hand. Participants washed their hands prior to expression as per ward protocol.

Two milk samples were collected from each mother on the following days:

i. Milk sample 1 (Macronutrient Analysis)

The first milk sample was collected on day seven of lactation;

^e Lactation consultants/ milk handlers: on-site personnel responsible for breastfeeding support and organisation of the on-site Human Milk Bank.

ii. Milk sample 2 (Micronutrient Analysis)

The second milk sample was collected on day nine of lactation.

Each sample contained a volume of 30-40ml of expressed breast milk for the analysis. Samples were collected after mothers expressed milk/ breastfed for her infant(s).

Mothers unable to provide the full sample (30-40ml) were asked to give 15ml on two consecutive days (i.e. 15ml on day seven and 15ml on day eight; or 15ml on day nine and 15ml on day ten of lactation). Refer to Figure 2.3 for a schematisation of the data collection procedures.

All milk samples were collected by the investigator or research assistant. Milk samples were stored at -20°C immediately after collection. Frozen samples were transported to the laboratories in a sealed cooler directly after removal from the freezer. The laboratories that performed the analysis included: Lactolab (Pty) Ltd (analysis of the macronutrients) and the Agricultural Research Council (ARC) Irene Analytical Services (analysis of the micronutrients).

The following nutritive components were analysed: total energy, carbohydrates, protein, lipids, phosphorus, iron, zinc and copper. The determination of macronutrients (lactose, protein and lipids) was done by means of infrared analysis (IDF 141B: 1996) with Bentley Fourier Transform Spectrometer (FTS) based on Fourier Transform Infrared Spectrometry Technology. The analyses were done according to international standards organisation (ISO) reference standards (described in section 2.13.1). Total carbohydrates were calculated by subtracting the protein and fat from the total solids. Thus: total carbohydrates (g) = total solids (g) – protein (g) – fat (g). The total energy of each sample was calculated by the following equation:

$$\text{Total energy (kCal/100ml)} = \frac{g \text{ protein} \times 17}{4.2} + \frac{g \text{ carbohydrates} \times 17}{4.2} + \frac{g \text{ fat} \times 38}{4.2}$$

The analysis of the micronutrients (phosphorus, iron, zinc and copper) was done by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Each sample was analysed three times whereupon the average was used.

Rationale behind breastmilk sample collection

Sampling was conducted on day seven and nine of lactation to ensure that lactation/ breastmilk production was established. Because breastmilk production is low in women who gave birth to premature infants, collection was done two days apart. Mothers who were not able to provide the full volume had the option of giving the full volume over two consecutive days per sample. Refer to Figure 2.3 for a schematic presentation of data collection procedures. The micronutrients selected for analyses (phosphorus, iron, zinc and copper) were selected for their role in growth and development and has been shown to be of the major minerals that are low in preterm infants. Refer to section 1.8.7 for a complete description of the importance of each mineral in the nutritional care of the preterm infant.

Breastmilk sample 1: Day 7	Breastmilk sample 2: Day 9
<ul style="list-style-type: none"> • Sample 1 (30-40ml OR 2x 15-20ml) • Macronutrients <ul style="list-style-type: none"> • Protein, carbohydrates, fat • Analyses at Lactolab 	<ul style="list-style-type: none"> • Sample 2 (30-40ml OR 2x 15-20ml) • Micronutrients <ul style="list-style-type: none"> • Phosphate, iron, zinc, copper • Analyses at ARC Irene

Figure 2.3: Schematic presentation of data collection procedures

2.11.2.4 Maternal anthropometry

Maternal anthropometric data [weight, height and mid-upper arm circumference (MUAC)] was collected on day seven after giving birth. Measurements were standardised and conducted according to the WHO Universal Standards.² Body Mass Index (BMI) was calculated according to the standard method, i.e. $BMI (kg/m^2) = \frac{weight}{height^2}$.

The following methods were used:

Weight:

A portable, electronic calibrated scale was used to conduct the measurements. Weight measurements were rounded off to the nearest 100g and were determined as follows:²

- i. The participant had to be wearing minimal clothing with her shoes taken off
- ii. Participant stood upright on the scale looking forward
- iii. The participant did not have support to stand
- iv. Weight was read
- v. Participant stepped off the scale
- vi. Procedure was repeated three times and an average value was calculated

Height:

A portable measuring stick (stadiometer) was used for height measurements. Height measurements were rounded off to the nearest 1mm and were determined as follows:²

- i. The participant stood barefoot on the ground with feet shoulder width apart and heels touching the back panel
- ii. All head gear had been removed
- iii. The participant stood upright with the spine and pelvis and shoulder level should touch the upright
- iv. Shoulders were relaxed and arms hung at the sides
- v. The measuring stick was lowered onto the participant's head and the measurement was taken with inhalation
- vi. Measurements were repeated three times and an average value was taken

MUAC:

A measuring tape was used to conduct the measurements. All measurements were rounded off to the nearest 1mm and was determined as follows:²

- i. The participant stood upright with hands hanging loosely at sides
- ii. Feet were shoulder width apart
- iii. The participant was asked to bend her right arm in a 90° angle with the palm facing upward
- iv. The distance between the acromium and olecranon was measured and the midpoint was found
- v. The participant was asked to relax her arm and to let it hang loosely at her side
- vi. The midarm circumference was measured by ensuring the measuring tape was parallel to the ground
- vii. It should not have been too loose and should not have cut into the participants' skin
- viii. The measurement was taken three times and the average measurement was calculated

2.11.3 Neonatal information

2.11.3.1 Establishing the gestational age

If the mother had not had an early sonar during pregnancy, gestational age was determined by the Ballard Score.^f [This method is used in the research setting (Kalafong Neonatal Unit) as early sonars are rarely available or adhered to.] The Ballard score was done at birth and repeated (checked) the first day when the most senior clinician was available. The Ballard score is accurate for the first five days of life. Thus, infants born over a weekend was scored on the first weekday after birth.

2.11.3.2 Neonatal anthropometric data

Neonatal anthropometric data [birthweight, recumbent length and head circumference (HC)] at birth was noted. The measurements were repeated according to standardised methods² to ascertain accuracy. Intra-uterine growth restriction was classified according to the Fenton growth chart for premature infants.³

^f Ballard Score: Technique to determine the gestational age of neonates whereby a score is assigned to various criteria. The sum of all scores is used to determine the gestational age. Criteria is divided into physical and neurological development areas.

Weight

The weight of each infant was determined as follows:²

- i. An electric scale was used, with an accuracy of 0.001kg;
- ii. All instruments were zero calibrated before commencing with the measurements;
- iii. Infants were weighed without any clothing or a nappy. If a nappy was worn, the weight was corrected by subtracting the weight of the nappy;
- iv. Infants were placed in the middle of the scale and the baby had to lie still before the reading was taken;
- v. The measurements were read to the nearest 0.001kg; and
- vi. The average of three measurements was reported.

Head circumference

Head circumference was taken with a standard measuring tape. Measurements were rounded off to the nearest millimetre and conducted according to the following method.²

- i. All head gear were removed
- ii. The measuring tape was wrapped around the widest part of the infant's head from the most prominent part of the head (approximately 1-2 fingers above the eye brows) to the point of the occipital bone (occipital bun)
- iii. Measurements were repeated three times and the average measurement was recorded.

Recumbent length

Length was measured with a portable, length board. Two persons were needed to do the measurement. All clothing were removed. The following procedures were used:²

- i. The infant was positioned with head touching the board. The infant was lying in a straight line. The Frankfort plane was perpendicular to the board. One person stood behind the infant's head and held the infant straight.
- ii. The second person stood next to the infant and hold the movable foot piece in his/her right hand. The infant's body was positioned so that the shoulders, back and buttocks were flat along the centre of the board. The infant's knees

were pushed down so that the legs were straight and flat against the centre of the board. The foot piece was brought to the infant's heel that formed a 90 degrees angle with the board.

- iii. Measurements were repeated three times and the average measurement was recorded.

2.12 Data Handling And Analysis

2.12.1 Laboratory techniques to analyse breastmilk samples

2.12.1.1 Macronutrients

i. Protein

The protein content of breastmilk was determined by measuring the absolute nitrogen content and then using a formula to calculate the total protein content. ISO 8968/ IDF 20 standards were used. The principle behind the procedure is to digest a part of the test proportion by using a block-digestion apparatus with a mixture of sulphuric acid and potassium sulphate and copper sulphate as a catalyst to convert organic nitrogen to ammonium sulphate.⁴ Analysis procedures were as follows:

- i. The test sample was prepared: Test sample was warmed in a water bath at 38°C. The test sample was mixed thoroughly by repeatedly inverting the test sample without frothing. The sample was cooled to room temperature before weighing.
- ii. Procedure: 12g potassium sulphate, 1ml copper sulphate solution, 20ml sulphuric acid and 5ml of the prepared sample was added to the clean digestion tube. The solution was then mixed.
- iii. Digestion: The digestion block was set at a low temperature (180- 230°C) to avoid foaming. The tube was transferred to the digestion block which was connected to a centrifuge. The rate of the centrifuge should have been sufficient to remove excess fumes. The mixture was digested for 30 minutes or until white fumes had developed. The process was continued until the solution was clear. The tube was removed from the block with the exhaust manifold in place. The tube was cooled down to room temperature over approximately 25 minutes. The cooled down solution was liquid

with a few crystals at the bottom. 85ml of water was added. The test tube was swirled so that all crystals dissolved.

- iv. Distillation: The condenser water for the apparatus was switched on. The digestion tube was attached to the distillation apparatus. A flask was positioned with 50ml boric acid under the the condenser. The steam distill method was used and 150ml of the solution was collected. The flask was removed from the distillation apparatus.
- v. Titration: The contents of the flask with the hydrochloric acid that formed during the distillation phase using a burett was titrated. The process was completed when the first traces of pink contents were distinguished. The reading was estimated to the nearest 0.05ml.
- vi. The nitrogen content to the nearest four decimal places was calculated.
- vii. The prude protein content was calculated and the the results were expressed to the nearest four decimal places.

ii) Lactose

Lactose content was determined by using the international standard: ISO 22662 /IDF 198 (2007).⁵ The procedures were as follow:

- i. Preparation of the sample: The test sample was warmed in a water bath between 38 and 40°C. The test sample was mized by inverting it gently and cooled down to 21°C.
- ii. Preparation of the standard solution: The appropriate amount of α -lactose monohydrate was weighed down to give 20mg/ml anhydrous α -lactose solution. The α -lactose monohydrate was then dissolved in 5ml High Performance Liquid Chromatography (HPLC) grade water. Added to this was 2ml of melezitose and mixed gently. The final α -lactose content was expressed in mg/ml. The solution was filtered through filter paper using a filter tunnel. The filtrate was then apsirated into a syringe.
- iii. Preparation of the test solution: The prepared test sample was weighed to the nearest 1mg into a 10ml volumetric flask and 5ml of the HPLC water that was prewarmed between 50 and 60°C was added. The solution was mixed until it presented homogenous. It was allowed to cool down to 20°C.
- iv. Prepare the filtrate: 2ml of the melezitose solution and 1.2ml of Biggs-Szijardo solution was added to the contents of the flask. It was mixed by gently inverting the

flask five times and then allowed to cool to room temperature for 10 minutes. The mixture was filtered by using filter paper then the mixture was transferred into a HPLC vial.

- v. HPLC determination: The detector was turned on at least 24 hours before the analyses. The internal temperature was set at 35.5°C and the flow rate of the HPLC water pump was controlled to 0.2ml/min for at least 20min while the column heater was set to room temperature. The latter was heated to 85°C then gradually increased to a flow rate to 0.6ml/min. Chromatographic conditions were strictly adhered to.
- vi. Calculations: Calculations were done by computer software which generated the lactose content as a curve by plotting the response ratio of lactose to a standard peak area. The software divided the concentration derived from the curve by the mass of the test sample to calculate the lactose mass as a fraction, expressed as a percentage.

iii) Fat

Fat content was determined by gravimetric analyses^g according to ISO 1211:2010(E) standards.⁶ The steps followed in the analyses are described below:

- i. Prepared the test sample: Using a warm waterbath, the test sample was warmed to a temperature of 38°C. If a homogenous sample was obtained, without prior warming, the test sample was brought to a temperature of 20°C.
- ii. Test portion: The test sample was mixed by gently inverting the bottle three to four times and immediately weighed to the nearest mg, 10-11g of the sample directly or indirectly into a fat-extraction flask.
- iii. Prepared a blank test (control): A blank test was carried out simultaneously with the determination by using the same reagents.
- iv. Prepared the fat-collecting vessel: The fat-collection vessel was dried with a few boiling aids in the oven at 102°C for 1 hour and then allowed to cool down in a metal container for 30 minutes. The fat collecting vessel was weighed to the nearest 1mg.

^g **Gravimetric analysis** describes a set of methods in analytical chemistry for the quantitative determination of an analyte based on the mass of a solid. A simple example is the measurement of solids suspended in a water sample: A known volume of water is filtered, and the collected solids are weighed.

- v. Determination: 2ml ammonia, 10ml ethanol and 25ml diethyl ether were added to the vessel and mixed well. The mixture was closed with a cork and shaken vigorously to prevent the formation of emulsions. 25ml of light petroleum was added and mixed for 30 seconds. The mixture was centrifuged for 1-5 minutes at a radial acceleration of 80-90g. The cork was removed and the mixture was then decanted into the previously prepared fat-collection vessel. The fat-collection vessel was heated to 102°C in an oven for 1h to dry. It was then removed from the heat and immediately verified if the fat emulsion was clear. Finally the mixture was allowed to cool to room temperature.
- vi. Calculation: The content of the fat in the sample was calculated and expressed as a percentage mass fraction.

2.12.1.2 Micronutrients

The following micronutrients were analysed: phosphate, iron, zinc and copper by means of the following procedure:

- i. Sample Preparation:

For the first 2 batches of milk samples (batch 2014-F-465, samples 1 to 25 and batch 2014-F-544, 29 samples from sample 026 to 064 with several gaps in the sample numbering) the liquid samples were digested and analysed directly with no initial sample preparation, just storage in a fridge. The third batch (batch 2015-F-117, samples 1 to 28) was freeze dried and milled by a different laboratory (ARC-Irene) to convert the liquid milk samples into milk powder samples. The milk samples were weighed before and after freeze drying to calculate the amount of water lost and the dry matter content of the liquid milk samples. The water or dry matter content values were used by ARC-Irene to convert the values for each element from the milk powder samples back to the original liquid milk samples.

- ii. Method for Perchloric and Nitric Acid Sample Digestion:

For the third batch, 0.5 g of milk powder sample was weighed off. For the first 2 batches, between 1.8 g and 4.4 g liquid sample was weighed off (see comment on masses below). The samples were digested with 7ml HNO₃ (conc. nitric acid) and 3ml HClO₄ (perchloric acid) at temperatures up to 180°C and brought to volume in a 100ml vol. flask. All samples were

digested in at least duplicate, mainly triplicate and for batch one some of the samples in quadruplicate.⁷

iii. ICP-OES Determination of P, Zn, Fe & Cu:

An aliquot of the digest solution is used for the ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometric) determination of P, Fe, Zn & Cu. The ICP-OES is a multi-element instrument. The instrument used (Varian Liberty Series II) is a sequential instrument, where the elements are determined almost simultaneously, with only a few seconds between each element. Each element is measured at an appropriate emission wavelength, chosen for high sensitivity and lack of spectral interferences. The wavelengths used were: P:213.618nm; Fe:259.94nm; Zn:213.856nm & Cu:324.754nm. Background correction was used for three of the elements, P, Fe & Cu, but unnecessary for Zn (low background and stronger signal for Zn).

The instrument is set up and operated according to the recommended procedures in the instrument manual. It is calibrated against a series of standard solutions, containing all the elements of interest in the proportions found in typical leaf samples. (Unpublished method developed by Mike Philpott at ARC-ISCW, based on the recommended procedures in the instrument manual.⁸

2.12.2 Demographic, medical, clinical and nutritional data

The demographic, medical, clinical and nutritional data were captured on a data collection form. Refer to Addendum A. The data collection form consisted of three sections:

i. Section 1

Information on demographics, obstetric history, breastfeeding history, HIV-status, treatment regimen and stage of disease of mother and infant. Refer to table 2.3 for information collected in section 1 of the information set.

Table 2.3: Information collected in section 1

Demographics	Maternal age Ethnicity Home language Income Number of people in household
Obstetric	Gestational age Gravida/ Para Multiparous pregnancy Previous premature deliveries
Breastfeeding history	History Problems
Maternal HIV information	HIV status CD4 count Treatment regimen Period of ART
Neonatal HIV information	HIV exposed ART

ii. Section 2

Information on medical history, current medical problems and medication (including ART) as well as clinical notes relevant to the investigation. In this part of the information form, spaces were provided for the researcher to note relevant information from the hospital file. Blood results and side room observations (vital signs, input-output charts) were noted where relevant.

iii. Section 3

Information on maternal and neonatal anthropometric measurements.
Refer to table 2.4 for information obtained from this section.

Table 2.4: Maternal and neonatal anthropometric information obtained from section 3:

Maternal Anthropometrics	Weight Height BMI MUAC
Neonatal Anthropometrics	Birthweight Length HC

iv. Section 4

To control/ensure all breastmilk samples were collected. A table was created for the researcher to evaluate if samples were collected to stipulated dates.

2.12.3 Classification of breastmilk composition

The absolute values of breastmilk composition were compared to results from other studies. Refer to table 1.3. The table was adapted from: Koletzko B,⁹ Anderson DM et al,¹⁰ Bishara R et al,¹¹ Bauer J, Gerss J,¹² Kim SJ et al,¹³ Ronayne de Ferrer PA et al,¹⁴ Tsang RC et al.¹⁵

2.12.4 Classifications of ART period

HIV-infected women on ART were classified according length of ART treatment. The National Consolidated Guidelines for the PMTCT of HIV states that HIV Testing and Counselling (HTC) should be done in three or more months before birth¹⁶ (assuming the infant will be born at term). Since the gestation period is interrupted in premature births, this guideline cannot be extrapolated to mothers who gave birth to premature infants. Therefore, the classification was setting specific, based on normal tendencies. The infants of mothers who received less than 4 weeks of ART were given donor breastmilk for the first 4 week of life.

i. < 4 weeks ART exposure

It is standard practice in the neonatal units of Kalafong Hospital to provide infants whose mothers received <4 weeks ART with donor breastmilk to avoid possible MTCT of HIV. In light of this, this period was classified as an insufficient treatment period.

- ii. 4-20 weeks ART exposure
- iii. >20 weeks ART exposure

2.12.5 Classification of nutritional status

2.12.5.1 Maternal nutritional status

Maternal undernutrition was defined as a BMI $<20.3\text{kg/m}^2$, height $<1.5\text{m}^2$ or MUAC $<5^{\text{th}}$ percentile.¹⁷ Currently there is no data to classify BMI of mothers who gave birth to premature infants. The WHO classification of an adequate nutritional status is a BMI of 20.3kg/m^2 one month postpartum.¹⁷ The researcher chose to use a maternal BMI of $\geq 20.3\text{kg/m}^2$ at birth as an indicator of a normal nutritional status, according to the WHO guideline.

BMI is an inaccurate parameter of nutritional status during pregnancy and lactation. MUAC is a potential indicator of nutritional status.¹⁸ A normal nutritional status was defined as MUAC 5-85th percentile. Overnutrition was defined as a MUAC $>85^{\text{th}}$ percentile. Measurements were plotted on the Centre of Disease Control and Prevention National Centre of Health Statistics' percentiles.¹⁹ Refer to Addendum E.

2.12.5.2 Neonatal nutritional status

IUGR was classified as a weight-for-age, length-for-age or HC-for-age $<10^{\text{th}}$ percentile on Fenton growth chart.² Refer to Addendum F.

2.13 Pilot Study

A pilot study was conducted to streamline all data collection procedures. All samples were collected during this period. Ten mother-infant pairs were recruited for a two-week period. Since the protocol was not affected or changed during the pilot study period, it was decided that the data collected in the pilot study could be used in the main study.

2.14 Data Capturing

Data was captured on Microsoft Excel 2013®.

2.15 Statistical Analysis

Data captured on Microsoft Excel 2013[®] was exported to Statistica version 12 (StatSoft Inc. [2015] STATISTICA [data analysis software system], www.statsoft.com) for data analysis.

Statistical analysis was done with the assistance of Tygerberg Biostatistics Unit, Stellenbosch University. Descriptive statistics were used to investigate the demographics, anthropometry and breastmilk nutritive composition of participants. When groups were compared, repeated measures of analysis of variance (ANOVA) and t-tests were used.

Confidence Intervals were determined in the analyses of data. A p-value of $p < 0.05$ was used to indicate a significant difference between groups.

2.16 Financial Disclosure

The investigation received funding from the Harry Crossley Foundation, administered by Stellenbosch University.

2.17 Ethics Approval

The study was granted written Ethics Approval from the Human Research Ethics Committee of Stellenbosch University (S13/09/165) and the Research Ethics Committee of the Faculty of Health Sciences at the University of Pretoria (191/2014). Guidelines for Applied Good Clinical Practice were adhered to at all times. The primary researcher completed the course for Applied Good Clinical Practice.

Participants were informed about the investigation in a language that they were fluent in and mothers received adequate time to consider participating. (Mother-infant pairs were identified at birth of the infant and mothers had seven days from birth to provide the first milk sample and to consider consent.) Written informed consent forms were available in Tswana, English and Afrikaans. (Refer to Addenda B-D). A copy was given to each mother to keep for her records.

It was considered standard medical care to conduct an ELISA test on all -uninfected mothers, however mothers had to provide verbal consent. Medical personnel who performed the test provided counselling as per usual protocol. Mothers who could not provide adequate volumes of breastmilk were not pressured to participate. No feeds were

withheld from infants. The sample volume of 30ml was stipulated as a minimum volume to analyse the samples by the laboratories.

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CHAPTER 3:

Results

RESULTS

The results section is reported in two separate articles. A degree of unavoidable overlap occurs between the information and data reported in the articles and the previous chapter. The articles will be submitted for publication to relevant peer reviewed journals.

3.1 ARTICLE 1

Differences in Breastmilk Composition of HIV-Infected and HIV-uninfected mothers of Premature Infants: Effects of Antiretroviral Therapy

3.2 ARTICLE 2

Anthropometric Parameters of HIV-Infected and HIV-Non Infected Mothers and their Premature Infants.

Differences in Breastmilk Composition of HIV-infected and HIV-uninfected mothers of Premature Infants: Effects of Antiretroviral Therapy

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ABSTRACT

A key strategy to prevent mother-to-child-transmission (MTCT) of the human immunodeficiency virus (HIV) and to reduce infant morbidity and mortality includes providing the HIV-exposed premature infant with breastmilk accompanied by maternal and infantile anti-retroviral therapy (ART). The effects of HIV and ART on premature breastmilk composition are largely unknown. The aim of the study was to assess and compare the breastmilk composition of HIV-infected mothers receiving ART and HIV-uninfected mothers who gave birth to premature infants.

Lactating HIV-infected women receiving ART (n=38) and HIV-uninfected women (n=36) who gave birth to premature infants were enrolled in the study. Mothers provided two breastmilk samples on day seven and nine of lactation. Breastmilk samples were analysed for total energy, protein, carbohydrates, fat, phosphate, iron, zinc and copper content.

Breastmilk of HIV-infected women contained higher protein (1.95 vs. 1.78 g/100g; $p=0.04$), fat (4.42 vs. 3.49 g/100g; $p=0.01$) and copper (0.64 vs. 0.56 mg/l; $p=0.02$) levels while carbohydrate (5.37 vs. 6.67 g/100g; $p=0.002$) and zinc (5.26 vs. 5.78 mg/l; $p=0.04$) levels were lower compared to those of HIV-uninfected women. Zinc levels were significantly lower in HIV-infected women with early gestation periods and the lowest levels were observed in women who received ART for ≤ 4 weeks (0.58mg/l; $p=0.03$). Total energy (78.22 vs. 61.48 kCal/100ml) and fat levels (5.39 vs. 3.00g/100ml) were significantly higher in the late gestation period HIV-infected women. Copper levels (0.61mg/l) were higher in late gestation period women who received >4 weeks ART exposure ($p=0.05$).

In this study differences existed in the breastmilk composition of HIV-infected women on ART compared to HIV-uninfected women. ART exposure period may influence breastmilk composition.

Keywords: Breastmilk, Human Immunodeficiency Virus (HIV) Antiretroviral Therapy (ART), Premature / Preterm Infant

Introduction

The heavy burden of maternal human immunodeficiency virus (HIV) infection is established in literature (Murry 2014, UNAIDS 2015, WHO 2015). As treatment has evolved into regimes of highly active antiretroviral therapy (HAART) the prevalence of low birth weight (LBW) and preterm birth among infants born to HIV-infected women has increased in developing countries (Watts 2013, van der Merwe 2011, Powis 2011, Kourtis 2007, Suy 2006).

It is well known that ART exhibit adverse outcomes in metabolic and endocrine systems in HIV-infected people. This includes dyslipidaemia, glucose intolerance and bone mineralisation abnormalities (Anuurad 2009, Da Cunha 2015, Abdollahli 2014, Riddler 2003). The effects of ART on breastmilk composition are however, unknown.

Proven to reduce morbidity and mortality, breastmilk is the recommended form of enteral nutrition for premature and LBW infants. Premature breastmilk composition is influenced by various factors which include: stage of lactation (Bauer 2011, Neville 1984, Bitman 1983, Emery 1978, Ogechi 2013), duration of lactation (Gidrewicz 2014, Hsu 2014, Luukkainen 1994), length of gestation (Brown 2008, Bokor 2007) and HIV-infection (van der Heever 2014). Limited data is available on the breastmilk composition of HIV-infected mothers who gave birth to preterm infants. Furthermore, no data is available regarding the effects of ART on breastmilk composition. A recent South African study found significantly higher protein and lower calcium levels in breastmilk samples of HIV-infected mothers when compared to HIV-uninfected mothers who gave birth to term infants (van der Heever 2014). The aim of this study was to assess and compare the breastmilk composition of HIV-infected mothers (receiving ART) and uninfected mothers who give birth to premature infants.

Materials and Methods

A cross-sectional descriptive study with an analytical component was conducted from the period August 2014 to April 2015 in the Neonatal Units of Kalafong Hospital, Pretoria, South Africa. Consecutive sampling was used to recruit participants. The study population comprised of postnatal lactating HIV-infected mothers receiving ART- and HIV-uninfected mothers who gave birth to premature infants. All mothers received accommodation at the unit as per standard protocol. The sample size determination was based on data of live

births from the institution. The sample size was calculated by a 1-way ANOVA calculation to obtain an effect size (RMSEE) = 0.55.

An HIV positive status was established by routine confirmed positive rapid HIV testing during the prenatal period and an HIV negative status was confirmed with routine ELISA testing in the postnatal period. The HIV-infected women were divided into three groups according to period of ART exposure: 1) <4 weeks of ART, 2) 4-20 weeks of ART and 3) >20 weeks of ART. It is practice in the unit to provide neonates whose mothers initiated ART at birth with donor breastmilk for the first 3-4 weeks. In light of this, a maternal ART exposure <4 weeks was classified as insufficient ART exposure and ≥ 4 weeks as sufficient ART exposure. Gestational age was estimated by using the Ballard score as early sonars are rarely performed. Premature infants born between 24-33 weeks gestation and 34-37 weeks gestation will be referred to as early- and late gestation periods respectively. Standard medical treatment was continued during the study period.

A researcher-administrated demographic questionnaire, tested for face and content validity during a pilot project, was completed. All personnel involved in the data collection were trained and standardised by the principal investigator.

Two breastmilk samples were collected from each mother. Breastmilk samples were collected on day seven and day nine of lactation for macronutrient and micronutrient analyses, respectively. Mothers expressed breastmilk with the manual expression technique. Thereafter, raw, untreated breastmilk samples were frozen at -20°C immediately after collection and transported to the Lactolab and Agricultural Research Council laboratories for macro-and micronutrient analyses, respectively.

The following nutritive components were analysed: total solids, ash, protein, carbohydrates, lipids, phosphorus, iron, zinc and copper. The determination of macronutrients (lactose, protein and fat) was done by means of infrared analysis (IDF 141B: 1996) with Bentley Fourier transform spectrometer (FTS) based on fourier transform infrared spectrometry technology (Griffiths 2007) according to international standards organisation (ISO) standards (ISO 2001, ISO 2007, ISO 2010). Total carbohydrates were determined by subtracting the determined protein and fat from the total solids. Total energy was calculated by adding the energy contributions of protein, carbohydrates and lipids. The

analysis of the micronutrients was done with inductively coupled plasma optical emission spectrometer (ICP-OES) (Boss 1997). Micronutrients, specifically those of importance in growth and antioxidant defences for premature infants, were selected for analysis.

Data captured on Microsoft Excel 2013[®] was exported to Statistica version 12 (StatSoft Inc. [2015] STATISTICA [data analysis software system], www.statsoft.com) for data analysis by the Tygerberg Biostatistics Unit, Stellenbosch University. Descriptive statistics were used to present the demographic characteristics of participants. When groups were compared, t-tests, ANOVA, Mann Whitney and Kruskal Wallis tests were used. The Bonferroni correlation test was performed when several dependent- or independent factors were analysed. Statistical significance was defined as $p < 0.05$.

The study was granted ethics approval from the Human Research Ethics Committee of Stellenbosch University (S13/09/165) and the Research Ethics Committee of the Faculty of Health Sciences at the University of Pretoria (191/2014). The study was conducted in accordance with Good Clinical Practice Guidelines.

Results

A total of 92 pairs were screened for inclusion in the investigation. Seventeen pairs were excluded of which twelve mothers were discharged before data collection procedures were completed, two mothers did not have adequate breastmilk volumes to provide a second sample, one mother was not on ART at the time of data collection and two mothers were excluded due to an ELISA not performed. One mother declined consent. The final sample consisted of 74 mother-infant pairs. This included 38 HIV-infected mothers and –exposed infants receiving ART; and 36 HIV-uninfected mothers and –unexposed infants. There were no significant differences between HIV-infected and HIV-uninfected women with respect to age, gestation period, ethnicity and education level (Table 1). The mean maternal age was 27.86 years ($SD \pm 5.57$) and the mean gestation period was 32.08 weeks ($SD \pm 3.03$). The majority (73.99%) of the participants were African women. Of the 74 participants, 38 (51%) were HIV-infected on ART and 36 (49%) were HIV-uninfected. All HIV-infected women received a fixed dose combination (FDC) ART which is a combination of tenofovir, emtricitabine and efavirenz (National Department of Health, South Africa 2014). Seven of the HIV-infected mothers (18%) received insufficient ART treatment for <4 weeks prior to

labour and 31 mothers (82%) were administered ART for >4 weeks. Of these, 13 mothers (34%) received treatment for 4-20 weeks and 18 mothers (47%) received treatment for >20 weeks. The period of ART ranged from one day to seven years with a mean period of 8.9 months (SD=15.4). In this group, the CD₄ count ranged from 51-720 cells/mm³ with a mean CD₄ count of 367 (SD=165) cells/mm³. No significant differences were found between gestational periods and ART duration (p=0.99 and p=1.00 respectively).

HIV-status and ART duration in relation to breastmilk composition

Breastmilk of HIV-infected women contained significantly higher protein (1.95 vs. 1.78 g/100g; p=0.04), fat (4.42 vs. 3.49 g/100g; p=0.01) and copper (0.64 vs. 0.56 mg/l; p=0.02) levels compared to HIV-uninfected women. The carbohydrate (5.37 vs. 6.67 g/100g; p=0.002) and zinc (5.26 vs. 5.78 mg/l; p=0.04) levels were significantly lower in HIV-infected women compared to HIV-uninfected women (Table 2). Differences were not detected in total energy, phosphate and iron levels with respect to HIV-status. The ART period did not affect the breastmilk composition (Supplementary material, Table 1).

HIV-status and gestation period in relation to breastmilk composition

Previous research had indicated that length of gestation significantly influences breastmilk composition (Gidrewicz 2014, Hsu 2014, Luukkainen 1994). Therefore, results were also analysed according to gestation period. Lower zinc levels were found in the early gestational age category (24-33weeks) with respect to HIV-exposure status (Supplementary material, Table 2). Lower zinc levels were observed in early gestation period HIV-infected mothers who received ART exposure for ≤4 weeks (p=0.03) (Figure 1). Total energy (78.22 vs. 61.48 kCal/100ml) and fat (5.39 vs. 3.00g/100ml) levels of late gestational age categories were significantly higher in breastmilk samples of women with insufficient ART compared to samples from HIV-uninfected women. Copper levels (0.61mg/l) were higher in women with late gestation who received 4 – 20 weeks treatment compared to HIV-uninfected women on ART (p=0.05) (Figure 2).

Discussion

Early recognition of HIV-infection is a key strategy in decreasing mother to child transmission. Even though no significant differences were found between gestational periods and ART duration it is evident that the current PMTCT programme is effective when comparing the mean ART treatment period and mean gestation periods.

Our results confirm data from a previous study conducted in South Africa that reported protein elevation in breastmilk of HIV-infected mothers of term infants. Furthermore, when they categorised the HIV-infected women into groups receiving ART and not, no statistical differences existed for all nutrients analysed (van der Heever 2014).

The effect of gestation period (prematurity), HIV-status and length of ART exposure on breastmilk composition presented intriguing results. When the sample was subdivided according to HIV status, the HIV-infected group receiving ART had significantly higher breastmilk protein, fat and copper levels, while carbohydrates and zinc levels were lower. The hypothesised mechanism underlying the observed differences may be due to the following explanations: i) HIV and/or ART exhibit changes in lactogenesis which alters breastmilk composition; or ii) breastmilk composition changes to accommodate the HIV-exposed infant's nutritional needs.

Analyses according to gestation and ART period illustrated that length of ART exposure had non-significant effects on breastmilk composition. Sub-analyses indicated that the elevation in total energy and fat was more predominant in the breastmilk of late gestation HIV-infected mothers with insufficient ART exposure. Fat significantly contributes to total energy and therefore the total energy is directly affected by the fat content. The physiological relevance of these findings remains unclear. However, proposed mechanisms supportive of the elevated breastmilk fat in women with insufficient ART exposure might be related to the normal rise in blood total cholesterol, low-density lipoprotein cholesterol (LDL) (Riddler 2003) and high-density lipoprotein (HDL) cholesterol observed after ART initiation (Baker 2011). It is hypothesized that these elevations in blood cholesterol affects the breastmilk composition of HIV-infected mothers during early treatment whereby increasing the total fat content of breastmilk.

Zinc is an essential micronutrient for optimal immune function (Dumrongwongsiri 2015), brain development and growth (Mathur 2015). Breastmilk zinc concentration was recently associated with low maternal zinc plasma levels (Dumrongwongsiri 2015). Low plasma zinc levels and inadequate zinc intake is prevalent in HIV-infected adults (Koch 1996; Carcamo 2006, Baum 1997, Beach 1992, Jones 2006). Our results support this finding as low breastmilk zinc levels were found in the HIV-infected mothers and more so for those who received insufficient periods of ART treatment (<4 weeks) and who gave birth to lower gestational age infants. Very low birth weight infants (VLBW), due to prematurity, are at particular risk for zinc deficiency which occurs mainly as a result of higher requirements combined with a low storage capacity (Jatinder 2004). The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) currently recommends intake of at least 1.1-2.0 mg/kg/day for all VLBW infants (Agostoni 2010). Wulf et al. showed that zinc deficiency was associated with lower gestational age and with being small for gestational age (Wulf 2013). Our study supports this finding. The impact of these findings is of importance as zinc deficiency in HIV-infected patients contributes to a compromised antioxidant defence system which may have adverse effects for the mother and infant (Cunningham-Rundles 2005). Several studies had shown low zinc levels among LBW infants which correlates with increased morbidity and failure to thrive (Mathur 2015; Friel 1994; Halas 1983). Supplementation with 2mg/kg/day zinc gluconate enhanced neurological developmental outcomes, such as alertness and attention pattern, and decreased hyperexcitability, and proportion with abnormal reflexes. (Mathur 2015).

Copper is required for the formation of enzymes, such as superoxide dismutase, which protects cells against oxidative damage, an outcome prevalent in prematurity. Neonates have low levels of ceruloplasmin, the transportation medium of copper, and its production only starts six to 12 weeks postnatally (Trindale 2005, Klein 2002, Agget 2000). The exact copper requirements for preterm infants are unknown; however, breastmilk provides adequate amounts (Trindale 2005, Reifen 1993). Interestingly, our results showed that HIV-infected women had higher breastmilk copper levels and the significance was predominantly in late gestation women who received ART 4-20 weeks. Copper is an acute phase reactant and therefore studies showed that serum copper levels were in the higher reference values in HIV-infected adults, especially in the early phase of infection

(Stambullian 2007, Moreno 1998). Consequently, copper levels are a helpful marker to determine the progression to Acquired Immunodeficiency Syndrome (AIDS) (Stambullian 2007, Moreno 1998).

Conclusions

In this study differences existed in the composition of premature breastmilk of HIV-infected and HIV-uninfected women. Significant deviations in breastmilk composition present with lower zinc and elevated fat in breastmilk of HIV-infected mothers after ART initiation which changes with progression of ART period. The variances in nutritive values in these milk samples do not, however, range outside the normal values of premature breastmilk composition. Further studies are warranted to confirm the differences and associated mechanisms in the composition of premature breastmilk of HIV-infected and HIV-uninfected women.

Key messages

Maternal HIV-infection and length of ART exposure exhibit changes in premature breastmilk composition. Breastmilk remains the first mode of enteral nutrition for premature HIV-exposed infants.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Contributions

CF, EvN and LdP designed the research study. CF performed data collection and analyzed the data. CF, EvN and LdP drafted the manuscript and reviewed the data; SD critically reviewed the paper. All authors read and approved the final version of the manuscript.

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TABLES

Table 1: Maternal Demographic characteristics according to early and late gestation

Characteristics	Sample (n=74)	Gestation: 24-33 weeks (n=41)			Gestation: 33-37 weeks (n=33)		
		HIV-infected (n=22)	HIV-uninfected (n=19)	p-value*	HIV-infected (n=15)	HIV-uninfected (n=18)	p-value*
Maternal Age (years), mean (SD)	27.86 (5.57)	29.77 (6.2)	26.74 (5.0)	0.10	27.80 (5.02)	26.78 (5.56)	0.59
Gestation (weeks) mean (SD)	32.08 (0.71)	30 (2.80)	30.16 (2.06)	0.84	34.53 (1.46)	34.61 (1.29)	0.87
Ethnicity				-----			1.00
African	73 (99%)	22 (100%)	19 (100%)		15 (100%)	17 (94%)	
Caucasian	1 (1%)	0 (0%)	0 (0%)		0 (0%)	1 (6%)	
Educational level				0.18			0.63
No formal education	14 (19%)	4 (18%)	6 (32%)		3 (20%)	1 (6%)	
> Grade 10	20 (27%)	7 (39%)	1 (5%)		5 (33%)	7 (39%)	
≥ Grade 12	25 (34%)	7 (39%)	7 (37%)		4 (27%)	7 (39%)	
Tertiary education	15 (20%)	4 (18%)	5 (26%)		3 (20%)	3 (17%)	

HIV: Human Immunodeficiency Virus

*Statistical significance $p < 0.05$

Table 2: Breastmilk composition of women who gave birth to premature infants according to HIV-status

Breastmilk composition (Reference values)†	All women Mean (SD) or median (IQR) (n=74)	HIV-infected on ART Mean (SD) or median (IQR) (n=38)	HIV-uninfected Mean (SD) or median (IQR) (n=36)	p-value*
Total energy (kCal/100ml) (67-78kCal/100ml)	68.23 (12.82)	69.82 (13.68)	66.54 (11.81)	0.27
Protein (g/100g) (1.6-2.2g/100g)	1.85 (1.67 to 2.15)	1.95 (1.73 to 2.28)	1.78 (1.59 to 1.98)	0.04*
Carbohydrates (g/100g) (6.7-7.3g/100g)	6.04 (1.98)	5.37 (1.75)	6.76 (1.21)	0.002**
Fat (g/100g) (3.5-4.8/100g)	3.97 (1.57)	4.42 (1.75)	3.49 (1.21)	0.01**
PO₄ (mg/l) (9.3-15.5mg/l)	16.11 (14.9 to 19.1)	16.00 (14.93 to 18)	17.3 (14.51 to 22.7)	0.16
Fe (mg/l) (0.9-3.7mg/l)	2.58 (1.8 to 4.0)	2.36 (1.8 to 4)	2.76 (1.75 to 4)	0.72
Zn (mg/l) (3.7-9.8mg/l)	5.41 (4.39 to 6.37)	5.26 (4.12 to 6.10)	5.78 (4.73 to 6.81)	0.04*
Cu (mg/l)	0.60 (0.16)	0.64 (0.15)	0.56 (0.15)	0.02*

(0.038-0.506mg/l)				
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HIV: Human Immunodeficiency Virus, ART: Anti-retroviral Therapy, IQR: Interquartile Range, PO₄: Phosphate, Fe: Iron, Zn: Zinc, Cu: Copper, Significance defined as *p<0.05; **p<0.01

†Reference values adapted from (day 7-21 of lactation): Koletzko B 2014, Anderson DM 1983, Bishara 2008, Bauer J, Gerss J 2011, Kim 2012, Ronayne 2001, Tsang 2005.

SUPPLEMENTARY MATERIAL

Table 1: Nutritional composition of premature breastmilk from HIV-infected women according to ART period

Breastmilk composition	HIV-infected on ART <4 weeks Mean (SD) or median (IQR) (n=7)	HIV-infected on ART 4-20 weeks Mean (SD) or median (IQR) (n=13)	HIV-infected ART>20 weeks Mean (SD) or median (IQR) (n=18)	p-value*
Total energy (kCal/100ml)	76.88 (10.61)	66.634 (15.42)	69.38(13.08)	0.28
Protein (g/100g)	2.08 (0.37)	2.11 (0.47)	1.91 (0.31)	0.32
Carbohydrates (g/100g)	4.85 (2.40)	5.35 (1.22)	5.58 (2.15)	0.70
Fat (g/100g)	3.49 (1.21)	5.40 (1.65)	4.32 (1.87)	0.24
PO ₄ (mg/l)	13.55 (11.6 to 15.5)	18.67 (12.19 to 19.25)	16.78 (15.2 to 18.95)	0.19
Fe (mg/l)	2.74 (1.52)	3.15 (1.70)	3.28 (2.33)	0.75
Zn (mg/l)	4.86 (1.86)	5.17 (2.57)	5.22 (1.31)	0.91
Cu (mg/l)	0.57 (0.15)	0.69 (0.15)	0.62 (0.15)	0.99

* Significance p<0.05. HIV: Human Immunodeficiency Virus, ART: Anti-retroviral Therapy, IQR: Interquartile Range PO₄: Phosphate, Fe: Iron, Zn: Zinc, Cu: Copper

Table 2: Breastmilk composition of mothers who gave birth to premature infants 24-33 weeks gestation according to HIV status and ART

*Significance defined as $p < 0.05$; HIV: Human Immunodeficiency Virus, ART: Anti-retroviral Therapy, IQR: Interquartile Range PO₄: Phosphate,

Breastmilk composition	Gestation: 24-33 weeks				
	HIV-infected (n=23)			HIV-uninfected Mean (SD) or median (IQR) (n=24)	p-value*
	ART <4 weeks Mean (SD) Mean (SD) or median (IQR) (n=4)	ART 4-20 weeks Mean (SD) or median (IQR) (n=8)	ART >20 weeks Mean (SD) or median (IQR) (n=11)		
Total energy (kCal/100ml)	75.86 (10.27)	61.85 (16.86)	68.68 (14.08)	70.16 (12.22)	0.34
Protein (g/100g)	2.01 (0.22)	2.25 (0.53)	1.93 (0.39)	1.99 (0.42)	0.28
Carbohydrates (g/100g)	4.66 (2.97)	5.85 (1.16)	5.04 (1.22)	6.76 (1.26)	0.12
Fat (g/100g)	5.4 (1.41)	3.57 (1.63)	4.21 (1.69)	3.84 (1.18)	0.18
PO₄ (mg/l)	13.56 (11.60 to 15.52)	18.67 (12.19 to 19.25)	16.79 (15.20 to 18.95)	20.68 (17.2 to 23.5)	0.17
Fe (mg/l)	2.55 (1.56 to 3.54)	3.27 (1.83 to 4.43)	2.28 (1.61 to 2.98)	3.95 (2.4-5.1)	0.11
Zn (mg/l)	4.03 (3.07 to 4.99)	5.15 (2.21 to 6.68)	5.44 (4.57 to 6.11)	6.83 (5.4 to 7.45)	0.03*
Cu (mg/l)	0.58 (0.12)	0.63 (0.12)	0.63 (0.19)	0.58 (0.16)	0.78

Fe: Iron, Zn: Zinc, Cu: Copper

Table 3: Breastmilk composition of mothers who gave birth to premature infants 34-37 weeks gestation according to HIV status and ART

Breastmilk Composiion	Gestation: 34-37 weeks				p-value*
	HIV-infected (n=15)			HIV-uninfected Mean (SD) or median (IQR) (n=15)	
	ART <4 weeks Mean (SD) or median (IQR) (n=3)	ART 4-20 weeks Mean (SD) or median (IQR) (n=5)	ART >20 weeks Mean (SD) or median (IQR) (n=7)		
Total energy (kCal/100ml)	78.22 (13.23)	74.30 (9.84)	70.46 (9.84)	61.48 (9.39)	0.03*
Protein (g/100g)	2.16 (0.56)	1.99 (0.28)	1.88 (0.28)	1.71 (0.44)	0.07
Carbohydrates (g/100g)	5.11 (1.96)	5.85 (1.16)	5.52 (2.14)	6.76(1.26)	0.20
Fat (g/100g)	5.39 (2.29)	4.75 (1.20)	4.48 (2.25)	3.00 (1.10)	0.04*
PO ₄ (mg/l)	15.27 (2.61)	17.55 (1.67)	15.66 (3.65)	14.88 (3.80)	0.52
Fe (mg/l)	3.00 (1.40-4.60)	2.96 (1.96 to 3.66)	4.86 (2.2 to 6.48)	2.44 (1.5 to 2.69)	0.20
Zn (mg/l)	5.97 (3.61 to 8.1)	5.21 (4.38 to 5.49)	4.87 (3.96 to 6.02)	5.28 (4.32 to 6.49)	0.76
Cu (mg/l)	0.56 (0.21)	0.80 (0.15)	0.61 (0.07)	0.52 (0.14)	0.05

* Significance defined as $p < 0.05$. HIV: Human Immunodeficiency Virus, ART: Anti-retroviral Therapy, IQR: Interquartile Range PO₄: Phosphate, Fe: Iron, Zn: Zinc, Cu: Copper

ARTICLE 2:

Anthropometric Parameters of HIV-infected and HIV-uninfected Mothers and their Premature Infants.

ABSTRACT

Background

Pregnancy outcomes, such as premature birth and intra-uterine growth restriction (IUGR) are associated with human immunodeficiency virus (HIV)-infection during pregnancy. Women with chronic undernutrition and obesity are more likely to give birth to IUGR premature infants. The objectives of the study were, to assess the maternal anthropometric parameters of HIV-infected and HIV-uninfected mothers. Furthermore this study aimed to determine the effects of anthropometric parameters, HIV status and the ART exposure on the anthropometric parameters of premature infants.

Participants and Methods

Study participants included HIV-infected and HIV–uninfected mothers who gave birth to premature infants. Maternal anthropometric data [weight, height and mid-upper arm circumference (MUAC)] were collected on day seven postpartum. Infant anthropometric data [weight, length and head circumference (HC)] were obtained at birth and length and HC were repeated on day seven postpartum. Demographic information was obtained through a questionnaire. Clinical information was obtained from the hospital files.

Results

The study population consisted of 74 mother-infant-pairs. This grouping comprised of 38 (51%) HIV-infected and 36 (49%) HIV-uninfected mothers and their premature infants. All HIV-infected mothers received ART. The mean maternal body mass index (BMI) was 26.7kg/m^2 ($\text{SD}\pm 4.88$) and MUAC was found to be within normal ranges (mean 28.9cm; $\text{SD}\pm 3.93$). Maternal underweight ($n=7$; 9%) and obesity prevalence was low ($n=7$; 9%). There was a high prevalence of IUGR ($n=40$; 54%). Neither maternal anthropometric parameters ($p=0.79$) nor HIV-status and ART regime ($p=0.72$) was associated with IUGR. Similarly, the nature of IUGR (symmetrical vs. asymmetrical) was not associated with maternal HIV-status ($p=1.00$). Head circumference restriction was lower in women with sufficient ART exposure periods ($p=0.003$).

Conclusion

Maternal anthropometric parameters, -HIV status and –ART exposure had no effect on the incidence of IUGR. Sufficient maternal ART exposure may positively influence head circumference at birth.

Introduction

A premature infant is defined as an infant born <37 weeks' gestation.¹ Fifteen million premature births occur worldwide of which 60% of these deliveries occur in low-and middle income countries.² Premature delivery is considered a significant global perinatal health problem with a rising incidence in Southern Africa.³ In a meta-analysis by Beck and colleagues, the worldwide prevalence of premature birth was 9.6% and 85% of these preterm births were from the African and Asian continent.³ Evidence from developing countries suggest that human immunodeficiency virus (HIV)-infected mothers have an increased risk of giving birth to premature infants and the occurrence is directly associated with clinical stage of disease.⁴⁻⁶ Research show that HIV-infected women were more likely to give birth to low birth weight (LBW) and intra-uterine growth restricted (IUGR) premature infants.^{2,5,6} The role of anti-retroviral therapy (ART) in IUGR is uncertain. Studies conducted in the developed world showed no association between *in utero* exposure to ART and IUGR,^{5, 7-9} however results obtained from the developing world are conflicting.⁹⁻¹²

Maternal anthropometric parameters during pregnancy are key factors in determining foetal growth and anthropometrical parameters at birth. In South Africa, poor household food security and the effects of HIV/AIDS Wasting Syndrome, or the combination thereof, may contribute to maternal undernutrition.¹³ In the undernourished mother, the supply of maternal- foetal nutrients are compromised, thereby restricting foetal growth. Foetal growth is more affected by chronic maternal undernutrition than nutrient restriction during the pregnancy period.¹⁴

In addition to undernutrition, the incidence of overweight and obesity is rising dramatically in South Africa.¹¹ In a small subset of people, maternal overnutrition may increase the risk of delivering a premature IUGR infant, though the exact mechanisms are poorly understood.¹⁵ An observational study found that obese women had a higher rate of IUGR deliveries as well as an increased frequency of admissions to the neonatal intensive care unit.¹⁶ Maternal overnutrition impedes placental growth and ultimately leads to growth faltering which significantly increases neonatal morbidity and mortality.¹⁷ Highly active antiretroviral therapy (HAART) use is often associated with overnutrition, metabolic- and endocrine abnormalities.¹⁸

The objectives of this study were to assess the maternal anthropometric parameters of HIV-infected and HIV-uninfected mothers as well as to assess of the neonatal anthropometric parameters of premature infants in relation to maternal anthropometric parameters (weight, height and MUAC), HIV status and ART regimen.

Methodology

A cross-sectional study with an analytical component was conducted in the postnatal wards of Kalafong Hospital, Gauteng, South Africa from August 2014 to April 2015.

Consecutive sampling was used to recruit participants. The study population included postnatal lactating HIV-infected mothers receiving ART and HIV-uninfected mothers who gave birth to premature infants. All mothers received accommodation in the ward as per standard protocol. The sample size was estimated by a statistician based on data of live births from the institution.

Consecutive consenting postnatal mother-premature infant pairs were included in the study. The study population consisted of HIV-infected and HIV-uninfected mothers who gave birth to premature infants. All HIV-infected mothers received ART. An HIV positive status was established by a routine confirmed positive rapid HIV test during the prenatal period and an HIV negative status was confirmed with a routine ELISA test in the postnatal period. Gestational age was estimated by using the Ballard score as early sonars are rarely performed. Standard means of medical treatment was continued during the study period.

A researcher-administered questionnaire, tested for face and content validity during a pilot project, was used in order to obtain demographic and clinical information from the mother. Information regarding HIV status, ART regimen and CD₄ count was collected. Maternal anthropometric information was obtained on day seven postnatally. This included: weight, height and the mid-upper-arm-circumference (MUAC). Maternal undernutrition was classified as a BMI <20.3¹⁸ and/or MUAC <214mm¹⁹ and maternal overnutrition was defined as a BMI >25kg/m².²⁰ Birth infantile anthropometric information was obtained from the patient folder. This included birth weight, -length and head circumference (HC). A trained dietitian repeated the length and HC measurements on day seven postnatally according to standardised procedures.²⁰ IUGR was defined as a weight, length or head circumference <10th percentile.¹ Symmetrical IUGR (s-IUGR) is defined as a weight, length and HC <10th

percentile and a ponderal index (PI) ≥ 2.0 and asymmetrical IUGR (a-IUGR) as weight $< 10^{\text{th}}$ percentile with the sparing of length and HC and a PI < 2.0 .^{22,23} Medical information were noted from the hospital file.

Data captured on Microsoft Excel 2013[®] was exported to Statistica version 12 (StatSoft Inc. [2015] STATISTICA [data analysis software system], www.statsoft.com) for data analysis. Statistical analysis was done with the assistance of Tygerberg Biostatistics Unit, Stellenbosch University. Descriptive statistics were used to report the demographics of participants. When groups were compared, ANOVA and t-tests were used. Statistical significance was defined as $p < 0.05$.

The study was granted ethics approval from the Human Research Ethics Committee of Stellenbosch University (S13/09/165) and the Research Ethics Committee of the Faculty of Health Sciences at the University of Pretoria (191/2014). The study was conducted in accordance with Good Clinical Practice Guidelines.

Results

A total of 92 mother-infant pairs were screened for inclusion in the investigation. Seventeen pairs were excluded of which twelve mothers were discharged before data collection procedures were completed, two mothers did not have adequate breastmilk volumes to provide a second sample, one mother were not on ART at the time of data collection and two mothers were excluded due to an ELISA test not performed. One mother declined consent. The final sample consisted out of 74 mother-infant pairs. Thirty eight (51%) mothers were HIV-infected and 36 (49%) were HIV-uninfected. All HIV-infected mothers received a fixed dose combination (FDC) ART which consist of tenofivir, emtricitabine and efavirenz.²⁴ The mean ART treatment period was 8.9 months (SD \pm 15.4; 1 day-7 years). In this group, the mean CD₄ was 367cells/mm³ (SD \pm 165; CI=51-720) cells/mm³. There were no significant differences in the demographic characteristics of mothers or infants. The mean gestational age of HIV-exposed infants were 31.8 weeks (SD \pm 3.2) and of HIV-unexposed were 32.4 weeks (SD \pm 2.86). The mean birthweight was 1468g (SD \pm 458.8g), length 40.7cm (SD \pm 5.0cm) and HC 28.7cm (SD \pm 3.1cm). The demographic characteristics are depicted in Table 1.

The mean maternal BMI was 26.7kg/m^2 ($\text{SD}\pm 4.88\text{ kg/m}^2$, $\text{CI}=25.7\text{-}27.9$), and the mean MUAC was 289mm ($\text{SD}\pm 3.93\text{mm}$; $\text{CI}=28.0\text{-}29.8$). Under- and overnutrition prevalence was low with 7 (9%) mothers classified as underweight and 7 (9%) as overnourished. Most mothers' ($n=59$; 80%) anthropometric parameters fell within normal ranges. The mean BMI and MUAC did not differ between HIV-infected and -uninfected mothers ($p=0.89$ and $p=0.71$ respectively). Furthermore ART exposure duration had no significant effect on maternal anthropometrical parameters (Figure 1).

IUGR was present in 40 (54%) of the premature infants. Of these, 13 (33%) were s-IUGR ($\text{PI}\geq 2.0$) and 27 (67%) were a-IUGR ($\text{PI}< 2.0$) indicating wasting at birth. Similar incidence of IUGR was found in infants born to HIV-infected and HIV-uninfected mothers. Furthermore, no significant differences ($p=1.00$) existed between infants with s-IUGR and a-IUGR according to maternal HIV status. Maternal anthropometric classifications ($p=0.79$) and maternal HIV-infection and -ART regimen ($p=0.82$) had no effect on the prevalence of IUGR (Table 2). No differences existed for birthweight ($p=0.18$) and length ($p=0.15$) or HC ($p=0.27$) between infants born to HIV-infected and HIV-uninfected mothers. HIV exposure did not influence birthweight ($p=0.18$), length ($p=0.15$) or HC ($p=0.27$). Maternal ART exposure time had no effect on infantile weights and lengths ($p=0.764$ and $p=0.647$ respectively), however infants of mothers who received ART for >20 weeks showed significantly less restrictions related to their head circumference measurements ($p=0.003$). Maternal ART exposure had no effect on birthweight categories (Figure 3). Similarly, maternal CD_4 cell counts had no effect on the incidence of IUGR.

Table 1: Demographic characteristics of the sample

Demographics	Mothers		
	HIV-infected n=38 (51%)	HIV-uninfected n=36 (49%)	p-value*
Ethnicity			p=0.3
African	38 (100%)	35 (97%)	
Caucasian	0 (0%)	1 (3%)	
Age in years mean (SD)	29 (5.57)	27 (5.11)	p=0.18
Education Level (%)			p=0.76
No formal education	7 (19%)	7 (18%)	
> Grade10	12 (33%)	8 (21%)	
≥ Grade 12	11 (31%)	14 (37%)	
Tertiary level	8 (22%)	7 (18%)	

*Significance defined as p=0.05

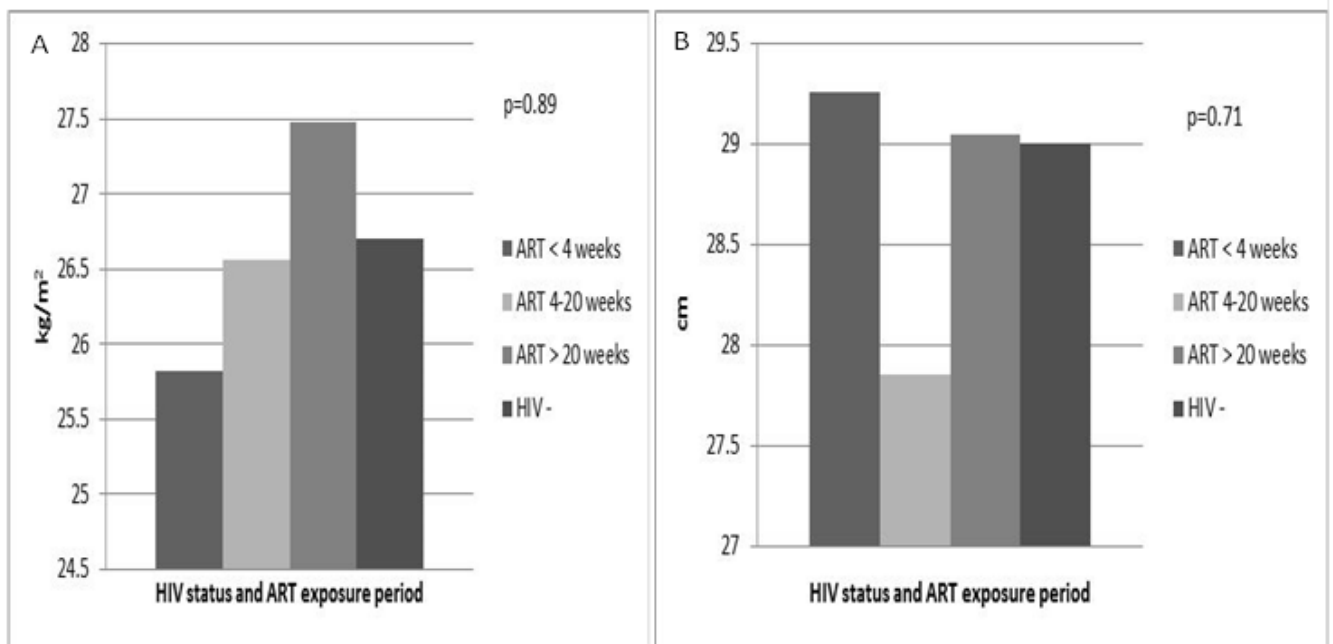


Figure 1: Maternal anthropometric data, -HIV status and ART exposure period. Maternal BMI (A) and MUAC (B). HIV: Human immunodeficiency virus, ART: Antiretroviral Therapy, IUGR: Intrauterine growth restriction, s-IUGR: symmetrical-IUGR, a-IUGR: asymmetrical IUGR. *p=0.05

Table 2: Neonatal anthropometric parameters according to maternal HIV status, - treatment regimen and –anthropometric parameters

Maternal Characteristics	IUGR (n=40)	Non-IUGR (n=34)	p-value*
	n (%)	n (%)	
HIV Status			p=0.72
HIV-infected on ART	20 (50%)	18 (53%)	
ART <4 weeks	5 (25%)	2 (11%)	
ART 4-20 weeks	7 (35%)	6 (33%)	
ART >20 weeks	8 (40%)	10 (56%)	
HIV-uninfected	20 (50%)	16 (47%)	
Maternal Status			p=0.79
Undernourished	3 (8%)	4 (12%)	
Normal	33 (82%)	27 (79%)	
Overnourished	4 (10%)	3 (9%)	

HIV: Human Immunodeficiency Virus, ART: Antiretroviral Therapy, IUGR: Intra-uterine Growth Restriction; *p=0.05

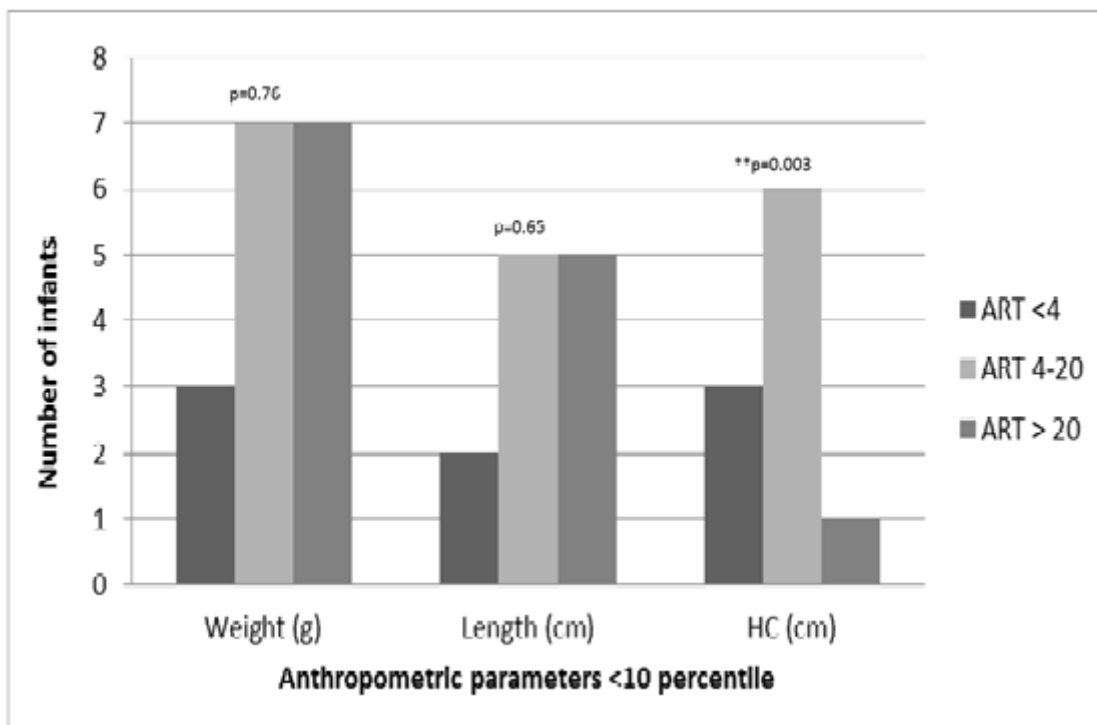


Figure 2: Anthropometric parameters <10 percentile of HIV-exposed premature infants according to maternal ART period. ART: Antiretroviral Therapy, HC: Head circumference, **p<0.01

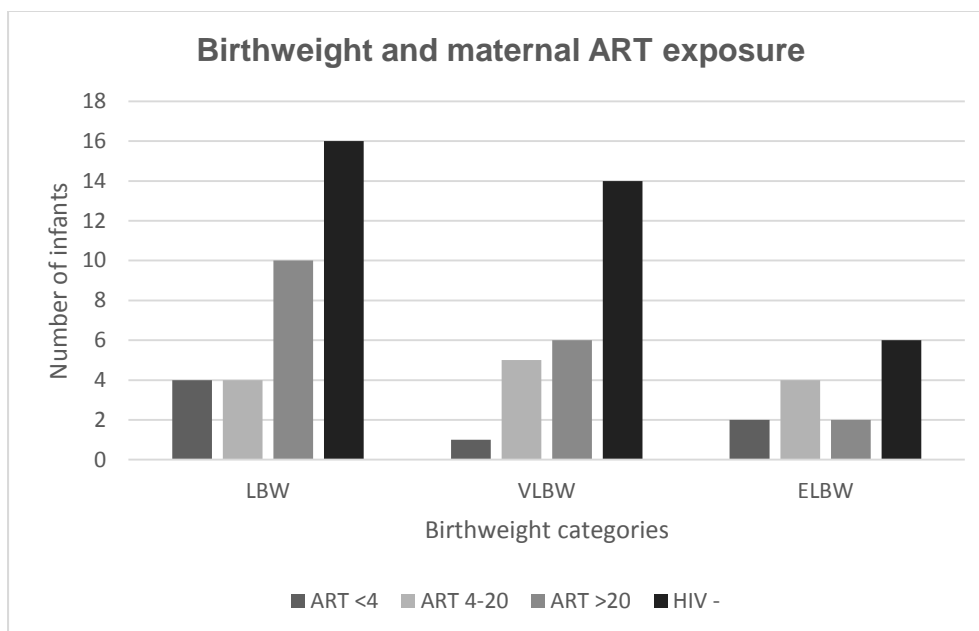


Figure 3: Birthweight according to maternal ART exposure time and HIV-uninfected mothers. ART: Antiretroviral Therapy, LBW: Low Birth Weight, VLBW: Very Low Birth Weight, ELBW: Extremely Low Birth Weight, p=0.63

Discussion

Early recognition of HIV infection is a key strategy in decreasing mother to child transmission. It is evident that the current PMTCT programme is effective when comparing the mean ART treatment period and mean gestation periods.

Our results did not show an association between maternal HIV-infection and adverse pregnancy outcomes, such as premature birth and IUGR. This is conflicting to numerous studies from Sub-Saharan Africa that reported significant results.⁴⁻⁶ The short- and long-term effects of ART on *in utero* development are unknown, particularly in developing countries. While some studies found that maternal antiretroviral drugs compromised neonatal anthropometric classification,^{5,7-9} others, including ours, did not.⁹⁻¹² Anthropometrics (birthweight, length and HC) and birthweight categories were not influenced by maternal HIV-status or –ART use. While ART may increase the risk for LBW,^{5,7-9} the importance of maternal ART provision is imperative to prevent MTCT of HIV. However, other strategies to prevent LBW should be optimised as LBW infants are at higher risk of mortality compared to a term appropriate for gestation infant.²⁵

Mothers had a mean BMI of 27.6kg/m² which suggests a risk for overweight among childbearing women. The prevalence of IUGR was high (53%) in this group. The combination of mothers at risk of overweight and infants being born with IUGR have important implications for HIV-infected and HIV-uninfected women of reproductive age as it indicates possible metabolic disturbances for them and their offspring. The role of ART may further disturb clinical anthropometric and metabolic parameters. ART is associated with metabolic aberrations, including central obesity, dyslipidaemia and insulin resistance.¹⁸ Although maternal anthropometrics were not influenced by ART exposure time, the possibility of pre-pregnancy- or future morphological and/or metabolic effects should not be excluded. Epidemiological studies and animal models associate LBW with risk of adult obesity and metabolic syndrome. Maternal overnutrition, whether from obesity, high fat diets or excessive weight gain in pregnancy, has delivered varied results concerning birth weight. However, in the adult offspring, obesity and metabolic abnormalities are highly prevalent indicating evidence of metabolic programming.²⁶⁻²⁹ The intrauterine exposure to endocrine disrupting chemicals (or obesogens), present in overweight mothers, alter the developmental programming of adipogenesis of the foetus through gene expression. The

developing foetus responds by producing structural and functional changes in tissues and organ systems, known as foetal programming, which result in increased plasticity of adipocytes.³⁰

A third (32%) of infants with IUGR presented with low HC measurements. Subanalyses demonstrated a higher incidence of HC restriction among women with ART exposure <20 weeks and insufficient ART exposure. This holds important implications as an impaired HC at birth is a strong indicator of impaired neurodevelopmental outcomes in childhood.³¹ Studies that explored prenatal exposure to ART did not find differences in infant neurodevelopmental outcomes.^{32,33} The results indicate the possibility that increased ART exposure might protect infants against impaired brain development, as seen in studies on longer ART duration and association with reduction of some neurologic impairment in children.³⁴

Conclusion

Maternal anthropometric parameters, -HIV status and -ART exposure showed no association with IUGR in this study. Sufficient maternal ART exposure may positively influence head circumference at birth.

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SUPPLEMENTARY MATERIAL

Table 1: Maternal Anthropometric parameters

Anthropometric Parameters	HIV-infected (n=38)			HIV-uninfected Mean (SD) (n=36)	p-value*
	ART <4 weeks Mean (SD) (n=7)	ART 4-20 weeks Mean (SD) (n=13)	ART >20 weeks Mean (SD) (n=18)		
Weight (kg)	65.84 (7.88)	66.12 (16.17)	69.89 (12.77)	67.60 (14.6)	0.86
Height (m)	1.60 (0.05)	1.58 (0.06)	1.60 (0.07)	1.59 (0.07)	0.86
BMI (kg/m ²)	25.82(3.63)	26.56 (6.93)	27.48 (4.71)	26.70 (4.43)	0.89
MUAC (mm)	292.60 (3.71)	278.50 (5.12)	290.50 (3.78)	290.00 (3.65)	0.71

HIV: Human Immunodeficiency Virus, ART: Antiretroviral Therapy, BMI: Body Mass Index, MUAC: Mid-upperarm circumference

* p=0.05

CHAPTER 4:

Conclusion and

Recommendations

CONCLUSIONS AND RECOMMENDATIONS

4.1 Summary of Study Objectives and Design

Premature delivery is considered a significant global perinatal health problem with rising incidence in Southern Africa.¹ HIV-infected women receiving ART are more likely to give birth to premature- and IUGR infants.² The current South African HIV infant feeding guidelines promote exclusive breastfeeding with dual provision of ART to HIV-infected mothers and HIV-exposed infants.^{4,5} The breastmilk composition of mothers of premature infants is highly variable and is influenced by factors that include stage of lactation,⁶⁻¹⁰ duration of lactation,¹¹⁻¹³ length of gestation,^{14,15} maternal anthropometric parameters¹⁶ and HIV-infection.¹⁷ Extensive literature exists to show that ART has various effects on metabolic and endocrine systems;¹⁸ however, the effect of ART on breastmilk composition is largely unknown. Recent research found differences in breastmilk composition between HIV-infected and HIV-uninfected women with term infants.¹⁷

The primary aim of the investigation was to assess and compare the breastmilk composition of HIV-infected mothers receiving ART and HIV-uninfected mothers who had given birth to premature infants. The objectives were as follows:

- i. To describe the HIV-infected mother population with reference to the use of ARV treatment schedule and disease state.
- ii. To compare the breastmilk components of HIV-infected mothers receiving ART, according to the ART regimen, in other words HAART and PMTCT, with those of HIV-uninfected mothers.
- iii. To assess the maternal anthropometric parameters (weight, height and MUAC) of HIV-infected and HIV-uninfected mothers.
- iv. To assess the neonatal anthropometric parameters of premature infants in relation to maternal anthropometric parameters (weight, height and MUAC), HIV status and ART regimen.

A cross-sectional study with an analytical component was conducted in the postnatal units of Kalafong Hospital, Pretoria; South Africa from August 2014 till April 2015. Mothers were included if: 1) they had given birth to a premature infant (24-37 weeks gestation); 2) were breastfeeding exclusively; and 3) if HIV-infected mothers received ART. Inclusion criteria of

infants were: 1) ≥ 24 -37 weeks gestational age (24-33 weeks were classified as earlier gestation and 34-37 weeks as later gestation); 2) were breastfed exclusively- either mother's own milk or donor breastmilk; 3) HIV-unexposed or HIV-exposed on prophylactic ART, 4) first born infant of twins.

The study population was divided into four subgroups for comparison:

- i. HIV-uninfected lactating women receiving ART who gave birth to premature infants 34-37 weeks gestation;
- ii. HIV-uninfected lactating women receiving ART who gave birth to premature infants 24-33 weeks gestation;
- iii. HIV-infected lactating women who gave birth to premature infants 34-37 weeks gestation;
- iv. HIV-infected lactating women who gave birth to premature infants 24-33 weeks gestation.
- v. First born infant of twins

The data of HIV-infected mothers were analysed according to length of ART exposure. ART exposure was seen as short treatment duration when ART was initiated <4 weeks before birth. Furthermore there was distinguished between ART treatment use 4-20 weeks and >20 weeks.

All mothers were encouraged to initiate breastfeeding or to express breastmilk within one day of giving birth. Breastmilk samples were collected on day seven and nine of lactation for macronutrient and micronutrient analyses respectively. Each mother provided two samples of 30ml breastmilk that had been expressed manually. A researcher-administered demographic questionnaire was completed, and medical/clinical information was obtained from the hospital file (refer to Addendum A). Maternal anthropometric measurement were performed on day seven postpartum. Infantile birth anthropometric information was obtained from the hospital file. The length and head circumference were measured on day seven of life. Gestation was classified by using the Ballard score in the absence of an early sonar. Normal standard of care was practised during the course of the study.

Breastmilk samples were analysed for total solids, ash, protein, lactose, fat, phosphorus, iron, zinc and copper. Total carbohydrates and total energy were calculated. All breastmilk

samples were raw, untreated samples, and therefore had not undergone any pasteurisation procedures.

The total study population consisted of 74 consenting mother-infant pairs. Thirty eight pairs (51%) were HIV-infected/ HIV-exposed and 36 (49%) pairs were HIV-uninfected/ HIV-unexposed.

4.2 Addressing the study objectives

In this section, each objective will be described and interpreted within the context of the results of the research.

4.2.1 Description of the HIV-infected mother population and ART use

ART is an effective way of reducing the viral load, increasing the maternal CD₄ count¹⁹ and minimizing the postnatal risk of MTCT of HIV through breastmilk.²⁰ When ART is available, MTCT of HIV can be reduced by 1–2%.²¹ The 2015 Consolidated National Guidelines for PMTCT and management of HIV in children, adolescents and adults recommend that all HIV-infected pregnant and lactating mothers receive ART for the duration of pregnancy and lactation. HIV-infected women are eligible for initiation of ART irrespective of CD₄ count.²² As of 2012, all HIV-infected pregnant women are initiated on FDC ART, which is a single-pill combination of tenovovir, emtricitabine and efavirenz.²²

In a recent retrospective descriptive study conducted at Kalafong Hospital, the cumulative incidence of HIV-infection at four weeks of life was found to be 25 out of every 1000 (2.5%) live births among VLBW^h infants. It is standard practice to provide infants with a single dose of nevirapine and raw breastmilk from the mother while providing mothers with ART.²³ The incidence of HIV-infection among these infants was comparable to statistics from Groote Schuur Hospital (incidence of 27 out of every 1000 at four weeks of life) where it is protocol to pasteurise breastmilk in addition to providing ART.²⁴ This data proves that raw breastmilk in conjunction with dual ART for mother and infant is a safe feeding option for premature infants.

It was not within the scope of this study to determine the prevalence of HIV-infection; however, a study conducted in the neonatal wards of Kalafong Hospital in 2005 indicated

^h VLBW infants are defined as infants born with a birthweight <1500g

that the prevalence of maternal HIV-infection was 26.3%,²⁵ and national data showed that 30% of women attending antenatal clinics were HIV-infected in 2008.²⁶

The results of this investigation indicated that all HIV-infected mothers received FDC ART. The CD₄ count of HIV-infected mothers varied widely (CI=51-720 cells/mm³) with mean CD₄ count of 367cells/mm³ (normal CD₄ range between 500- and 1500 cells/mm³).²⁷ Seven (10%) mothers had received ART for <4weeks. It is practice in Kalafong Hospital to provide infants whose mothers received <4 weeks ART with donor breastmilk for the first 3- 4 weeks of life. In light of this practice, the researchers viewed this period as insufficient time to prevent MTCT of HIV. The period of ART exposure elicited fascinating results with regard to breastmilk composition. It did not affect maternal anthropometrics, but sufficient ART exposure showed a positive effect on HC in the infants. Details regarding breastmilk composition and anthropometrics will be discussed in section 4.2.2 and 4.2.3 respectively.

4.2.2 Breastmilk Composition of HIV-infected Women receiving ART

H₀: There is no difference between the breast milk composition of HIV-infected mothers receiving ART and HIV-uninfected mothers who gave birth to a premature infant.

There is a high incidence of premature birth among HIV-infected women which associates directly with disease staging.^{2,28,29} A recent African study showed that 19.1% of premature births are from HIV-infected women.³⁰ With the advancement of ART regimes, an increase in prematurity has been observed in developing countries.³¹⁻³⁴ It is well known that ART exerts potent metabolic- and endocrine side-effects such as dyslipidaemia, glucose intolerance and bone mineral abnormalities¹⁸ with several metabolic aberrations occurring soon after ART initiation.^{35,36} However, the effects of HIV-infection and ART on breastmilk composition is unknown. The literature is deficient in studies that investigated the compositional differences in breastmilk between HIV-infected and –uninfected women. Recently, De Wet et al demonstrated differences between HIV-infected and HIV-uninfected women from Mangaung who had given birth to term infants.¹⁷

The results of this study indicated significant differences between HIV-infected women receiving ART and HIV-uninfected women who had given birth to premature infants. Protein, fat and copper levels were higher while carbohydrates and zinc were lower in HIV-infected women compared to HIV-uninfected women. Differences were more prominent

when groups were divided according to HIV status alone, than when groups were divided according to HIV status and gestation duration. Interestingly, all compositional factors were not consistently higher or lower in HIV-infected women receiving ART, in other words, some nutrients were higher and others were lower when compared to HIV-uninfected women's breastmilk.

The results demonstrated that breastmilk samples with a higher fat content was predominantly from late gestation HIV-infected women with ART exposure <4 weeks. Very few studies have investigated the metabolic and/or nutritional effects soon after ART initiation. Studies have shown that there is a rapid increase in total cholesterol, HDL cholesterol and LDL cholesterol.^{35,36} Considering the results of the investigation, it seems as if there is a dramatic metabolic deviance soon after ART initiation that possibly also influences breastmilk composition.

The significance of the lower zinc levels observed among HIV-infected women was predominantly from early gestation period women with <4 weeks ART exposure. Zinc is of great importance in growth, and brain development.³⁸ The impact of these findings are important since zinc deficiency in HIV-infected patients contribute to a compromised antioxidant defence system which may have adverse effects for the mother and infant.³⁹

Copper acts as an essential cofactor in many enzymes involved in metabolic reactions, such as angiogenesis, oxygen transport and antioxidant defences.^{40,41} Pregnancy induces a sudden rise in plasma copper levels which return to pre-pregnancy values shortly after delivery, partly as a result of ceruloplasmin synthesis. Ceruloplasmin is a copper-binding protein which is influenced by oestrogen⁴² and is a major acute phase reactant.⁴³ Differences in copper levels were found among late gestational women with ART exposure of 4-20 weeks, possibly indicating higher stress levels among this group. HIV-infection is associated with increased inflammation levels, opportunistic infections, AIDS-associated malignancies and several non-infectious conditions. These conditions often become visible shortly after the initiation of ART.⁴⁴

There is a deficit in studies conducted on the breastmilk concentration of HIV-infected women with preterm infants. It is however important to keep in mind that serum levels of HIV-infected women cannot be extrapolated to breastmilk concentrations. Although

differences were found, the variances in nutritive values in these milk samples did not, however, range outside the normal values of premature breastmilk composition. Further studies are warranted to confirm the differences and associated mechanisms.

The results demonstrated intriguing results on the contribution of ART exposure in breastmilk composition, however the contribution of disease stage (or degree of immunosuppression) remain to be explored.

Interestingly, a linear incline or decline was observed for all nutrients with ART exposure period. Although differences existed, the composition values still fell into the upper-or lower range observed in other studies.⁴⁵⁻⁵¹ It is well-known that preterm breastmilk does not fully meet the nutritional requirements of premature infants and needs to be fortified.⁵¹

Literature shows that breastmilk is not influenced by maternal nutritional status, except in severe starvation conditions.⁵³ The researchers did not account for this in the results due to the following reasons:

- i. Only anthropometric and clinical data were collected. In order to obtain a complete picture of nutritional status, biochemical, clinical and dietary information are also needed.
- ii. The number of mothers that were classified as under- and overnourished were low [n=7 (9%) and n=7 (9%) respectively].

The null hypothesis is rejected. A distinct difference was found in the nutritive breastmilk composition of HIV-infected mothers receiving ART and that of HIV-uninfected mothers who gave birth to a premature infant, bearing in mind that the values were within the normal range of recorded premature breastmilk composition.

4.2.3 Maternal Anthropometric parameters of HIV-infected women

H₀: Maternal anthropometric parameters, HIV status and ART regime do not affect neonatal anthropometric parameters at birth

Although HIV-infection is not associated with adverse birth outcomes in first world countries,^{2,53} several African studies have shown an increased incidence of prematurity, LBW and IUGR among HIV-infected women.^{2,28} It is difficult to demonstrate the relative

contribution of HIV to these parameters due to small samples and a lack of HIV-specific data from the available studies. Moreover, studies included different subtypes of HIV. In South Africa, the clade C subtypeⁱ is most predominant which is associated with increased MTCT of HIV and progression to AIDS.² Progression of HIV disease increases the risk of delivering IUGR infants.^{2,28,53-55} The role of HAART in IUGR is uncertain. Studies conducted in the developed world showed no association between *in utero* exposure to HAART and IUGR,^{28,29} but results obtained from the developing world are conflicting.⁴⁹⁻⁵⁵ Recently, efavirenz-based ART regimens were shown to correlate with an increase in adverse birth outcomes, including birth defects, stillbirths, abortion, LBW and prematurity.⁵⁶

Chronic undernutrition, often resulting from HIV infection, leads to foetal growth restriction and SGA infants.⁵⁷ On the other side of the malnutrition coin, the incidence of overweight and obesity is rising dramatically in South Africa.⁵⁸ In a small subset of people, maternal overnutrition may increase the risk of delivering a premature IUGR infant, though the exact mechanisms are poorly understood.⁵⁹ Obesity is often associated with chronic diseases of which the effects on birth outcomes are unquantified.⁶⁰ Furthermore, many research studies are investigating the effects of metabolic programming on the offspring of overweight and obese women. Exposure to endocrine disrupting chemicals causes adverse genetic changes that passed on from mother to fetus, which in turn predisposes the infant to obesity and chronic diseases later in life.⁶¹

The results of this study indicated a high prevalence of IUGR (53%) in the study population. Gilfillan and colleagues explored the characteristics of African women who had given birth to premature IUGR infants at Kalafong Hospital. The results indicated that maternal age, nutritional status, birth spacing, smoking and alcohol use during pregnancy as well as hypertension during the last trimester contributed to IUGR birth.²⁵ The PI, defined as weight (g) / length (cm)³, is a simple and valuable method to identify wasting or disproportionate IUGR at birth. A PI of <2.0 is defined as low in premature infants. A large proportion (67%) of infants had a low PI. Poor weight gain with the preservation of length and/or severe nutritional insults in the third trimester leads to a low PI. On the other hand, a third of infants presented with s-IUGR. Chronic maternal undernutrition and the presence of

ⁱ Clade C subtype: The strain of HIV-1 into four groups, namely groups M, N, O and P. In the M-group several subtypes/clades of HIV can be distinguished. Subtype/clade C is predominantly found in Southern Africa and is associated with an increased disease progression compared to clade A.

untrauterine infections are associated with s-IUGR. The high incidence of IUGR in this population has serious future health consequences. No prospective studies had been conducted in our population, but data links IUGR with chronic diseases (coronary heart disease, Type II Diabetes Mellitus, hypertension and stroke) in adulthood.⁶²

The results of this investigation indicated that maternal HIV-status and CD₄ count were not associated with IUGR; therefore, other maternal factors (including the factors mentioned above) could have contributed to this occurrence. Furthermore, there was no association between maternal anthropometric parameters and IUGR in its entirety. This might be due to the relatively low prevalence of underweight (9%) and overnourished mothers (9%) in the sample. In addition, the type of IUGR was not affected by the maternal HIV status and ART regimen. A significant association existed between maternal duration of ART treatment and head circumference restriction at birth. A higher incidence of head circumference restriction was found among women with ART exposure <20 weeks. This holds important implications as an impaired head circumference at birth is a strong indicator of impaired neurodevelopmental outcomes in childhood.⁶³ Studies that explored prenatal exposure to ART did not find differences in infant neurodevelopmental outcomes.^{64,65} The results indicated the possibility that increased ART exposure protects against impaired brain development. This finding corresponds with studies which found that a longer ART duration was associated with reduction of some neurologic impairment in children.⁶⁶

The null hypothesis that states “maternal anthropometric parameters, HIV status and ART regime do not affect neonatal anthropometric parameters at birth” is therefore partially accepted.

4.3 LIMITATIONS OF THE INVESTIGATION

The following limitations may have influenced the results of the investigation:

- i. The initial goal was to include 25 mother-infant pairs in each of the four subgroups in order to obtain an effect size (RMSEE) of 0.45. However, due to slow enrolment of the HIV-infected groups, only 17 mother-infant pairs were included in each of the four subgroups, giving an effect size (RMSEE) of 0.55. The proportionate prevalence of HIV-exposed versus HIV-unexposed infants led to

lower patient numbers and a strenuous data collection period with financial constraints.

- ii. When it was not possible to take the infant's length on a length board (e.g. intubated infants), a non-stretchable measuring tape was used which is not as accurate as a length board.
- iii. Although corrected for, some mothers presented with oedema which may have influenced precision of the anthropometrical measurements. BMI was however, adjusted to correct for oedema.
- iv. A high number of non-South African patients are admitted to Kalafong Hospital. This might have led to variability in the study population.
- v. Mothers who gave birth to twins were included in the study (n=9). The first born twin was included. Breastmilk values may be different to mothers who deliver to singleton infants. Twins frequently have a shorter gestation period and lower birth anthropometrics.
- vi. Low percentages of underweight and obese mothers were present in the study population. Therefore, it was difficult to show significant associations regarding their anthropometrics and IUGR of their infants.

The following data could have strengthened the results of the study:

- i. *Blood tests: total protein, albumin, pre-albumin and C-reactive protein, urea and creatinine*: useful measurements to determine nutritional status and levels of stress (acute phase response).
- ii. *Dietary intake (e.g. food frequency questionnaires, 24-h recall, and 3-day diet history)*: To strengthen nutritional status classifications and determine effect on certain limited parameters of breastmilk composition.
- iii. *Infantile PCR at birth and four weeks postpartum*: To determine the MTCT of HIV.
- iv. *Growth velocities of IUGR HIV-infected, HIV-uninfected but exposed and –uninfected infants at 6 months*: To determine if there was a correction in SGA infants and draw associations according to HIV-status/exposure. Associations can be made between breastmilk composition and growth.
- v. *Maternal pregnancy weight gain*: It was not possible to determine an association between a low PI and poor pregnancy weight gain.

4.4 RECOMMENDATIONS:

Recommendations to address the research question include:

- i. According to the researchers' best knowledge, this was the first study that found differences in breastmilk composition between HIV-infected and HIV-uninfected mothers with premature infants. More studies should be conducted to confirm the differences and explore other nutrients that make up the composition of preterm breastmilk.
- ii. A full analysis of micronutrients and trace elements in breastmilk samples will provide a more complete depiction of differences in nutritive composition.
- iii. A larger subsample of underweight and obese participants should be included to adequately investigate the influence of maternal anthropometric parameters on that of the infants.
- iv. Although the researchers were able to prove significant differences in breastmilk composition among the subsamples, the results could be strengthened with larger sample sizes.

Recommendations for further research include the following:

- i. The researchers specifically chose to work with the Ballard score, because neonatal nutritional parameters needed to be plotted (and very few women have access to an early sonar). However, future studies that investigate breastmilk composition only, should rather use birthweight cut-offs.
- ii. Because all mothers included in the study were lodgers and received three meals from the hospital's food service unit, it was assumed that dietary intake was comparable for all mothers and therefore dietary intake was not investigated. Future studies could take the habitual diets of HIV-infected and HIV-uninfected mothers into account.
- iii. Non-nutritive (immunological) factors should be compared between HIV-infected and HIV-uninfected mothers with premature infants. Non-nutritive factors may also contribute to the macronutrient composition of breastmilk, for example IgA and lysozyme, may contribute to total proteins and oligosaccharides may contribute to total carbohydrates.

- iv. **Macronutrients:** The study found elevation of protein and total fat in preterm breastmilk of HIV-infected women. The exact proteins and fatty acids that contribute to the elevation should be further explored. Carbohydrates were lower in the HIV-infected group. The breakdown of sugars and other carbohydrate-containing substrates should be analysed and compared between HIV-infected and HIV-uninfected women.
- v. **Trace-elements:** Copper was elevated and zinc decreased in HIV-infected women. Research may invest in the raised anti-oxidant properties of HIV-infected breastmilk as well as the copper-zinc interaction. Furthermore, the role of zinc in retarded growth of HIV-infected breastfed infants should be explored further.
- vi. Investigations should be conducted to explore the compositional differences with respect to nutrients not investigated in this study.
- vii. A high prevalence of IUGR was found in the infant study population. The study could not draw conclusive associations between maternal anthropometric parameters and ART use; therefore, other medical, maternal and social factors should be explored.
- viii. Prospective studies should investigate longterm outcomes such as growth and cognitive development of HIV-exposed infants, especially those born with head circumference restriction.
- ix. The metabolic programming hypothesis should be explored in this population, in other words SGA infants born to mothers at risk of overnutrition.
- x. The micronutrient status of mothers should be explored in relation to breastmilk composition and risk of IUGR.
- xi. The role of disease stage in breastmilk composition should be explored.

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ADDENDA

ADDENDUM A: QUESTIONNAIRE**Demographic Questionnaire**

Participant number	
1. DOB	

2. Maternal ethnicity:	
a) African (Black)	b) Caucasian (White)
c) Coloured (Mixed ancestry)	d) Asian
e) Indian	f) Other, specify

3. Home (primary) language:	
a) English	b) Afrikaans
c) Xhosa	d) Zulu
e) Tswana	f) Southern Sotho
g) Northern Sotho	h) Venda
i) Tsonga	j) Swati
k) Ndebele	l) Other, specify

4. Maternal level of education:	
a) No formal level of education	b) Highest grade completed
c) Tertiary level	d) Other (specify)

5. Monthly income:	
a) Number of working adults	
b) Grant(s) (Y/N) and amount	
c) Average monthly income	

6. Number of people in household:	Adults:
	Children:

7. Age of infant at birth (weeks gestation) :	
8. Parity (including this pregnancy): Para/Gravida	
9. Multiparous (Y/N):	
10. Has the mother breastfed an infant before? (Y/N) If yes, how many infants?	
11. List problems that prevented breastfeeding previously OR reasons for choosing formula milk.	
12. Has the mother given birth to a premature infant before?	

13. HIV status:	
a) HIV-infected receiving HAART (Y/N)	
b) If yes to (a), list ARV's and dosages	
c) CD ₄ count	
d) When was last CD ₄ count taken?	i) Before pregnancy of this infant
Date: if available	ii) During pregnancy of this infant
	iii) After birth of this infant
e) If no to (a), rapid HIV test result (pos/neg)	
Infant receiving treatment? (Y/N)	

14. List chronic medication and dosages:

--	--

15. Medical history/ Clinical information:

--

Breastmilk samples obtained:	Date:	Mark if received	
Breastmilk samples 1:		Sample 1	Sample 2
Breastmilk samples 2:		Sample 1	Sample 2

MATERNAL ANTHROPOMETRIC INFORMATION	
Date	
Weight	
Height	
BMI	
MUAC	
Oedematous? (Y/N)	
If yes, grade?	
NEONATAL ANTHROPOMETRIC INFORMATION	
DOB	
Date of collection	
Weight	
Length	
HC	

ADDENDUM B: ENGLISH CONSENT FORM

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Nr:

TITLE OF THE RESEARCH PROJECT:

Breastmilk Composition of HIV-infected Mothers Receiving Anti-retroviral Therapy who Gave Birth to Premature Infants

REFERENCE NUMBER:

S13/09/165

PRINCIPAL INVESTIGATOR:

Carike den Boer

ADDRESS:

University of Stellenbosch

CONTACT NUMBER:

072 690 6990 (Carike den Boer)

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research is about and how you could be involved. Also, your participation is **entirely voluntary** and you are free to say no to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and Kalafong Hospital. It will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

The researcher will ask you if she can look in your hospital file to get information on your medical background and your HIV status. All information will be kept confidential and private. To get information about your nutritional well-being you will be weighed and your height and upper arm circumference will be measured. A translator might be present to help the researcher with the procedures. Then, you will be asked to express a little bit of your breastmilk into a container. One sample of 30ml each will be taken on the seventh day from when you started to produce breastmilk and the other sample (also 30ml) nine days from when you started to produce breastmilk. It is anticipated that a total of a 100 mothers will participate in the study at Kalafong Hospital.

What will happen to me if I do take part?

You will be responsible to give the researchers honest and true information regarding yourself and your health. You will be asked to give the researchers one sample of your breastmilk on two occasions. You will also be weighed and measured.

The researcher will also measure your baby's weight, length and head circumference or get it from the hospital file.

Breastmilk: As part of the study, we want to measure some of the ingredients in your breastmilk, such as protein, sugars, fat and minerals.

These milk samples will be frozen before being tested in the laboratory. The sample will be collected in a clean container free of germs.

Information from your hospital file will be used in the study such as how your baby was born, your HIV status and what medications you received during your pregnancy. All pregnant women in Gauteng are tested for HIV during pregnancy and because it is important for us to know your HIV status, we will get that information from your hospital folder.

Why have you been invited to participate?

You have been chosen to take part in the study because you gave birth to a preterm baby and are currently breastfeeding or providing breastmilk to your infant.

What will your responsibilities be?

You will be responsible to provide the researchers with honest and true information regarding yourself and your health. You will be asked to give the researchers two samples of your breastmilk. You will also be weighed and measured.

Will you benefit from taking part in this research?

You will not directly benefit from participating in this study, but will not be disadvantaged by not participating. Your medical care during your hospital stay will not be different from other women (who are participating or who are not participating in the study). The results of the group as a whole will be published or presented to other healthcare workers, but without mentioning your name. Other health care professionals (such as doctors, dietitians and nurses) can do further research using the results of this study, that might, in future, have benefits for babies who are breastfed.

Are there in risks involved in your taking part in this research?

No, you are not at risk of any harm.

Who will have access to your medical records?

The researcher will have access to your medical records. All information will be kept confidential (secret). The results of the group as a whole will be used in an article that will be published in a medical journal. Your name will not be used- you will remain **anonymous**.

The supervisors to the researcher may also have access to the information. This information will be kept confidential (secret).

Will my participation in the study be kept confidential?

Yes, your participation will be kept confidential. The milk samples will be destroyed after the study is completed. The milk samples will be numbered and will not be tracked back to you. Only the researcher will know which samples belong to which mother. The researcher will

keep all your information strictly confidential and no information will be given about you to any person who is not directly associated with this study.

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

It is unlikely that something will go wrong. If you cannot give the researcher a breastmilk sample or stand up to be measured, the researcher will ask you again at a later time.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study. There will be no costs involved for you, if you do take part.

Is there anything else that you should know or do?

You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by the researcher.

You can also contact the principal researcher, Carike den Boer, at any time at 072 690 6990 if you have any questions regarding the study.

You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I agree to take part in a research study entitled

Breastmilk Composition of HIV-infected Mothers Receiving Anti-retroviral Therapy who Gave Birth To Premature Infants.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) on (*date*) 2014.

.....

Signature of participant

.....

Signature of witness

Declaration by investigator

I (*name*) declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above

- I did/did not use a interpreter. (*If a interpreter is used then the interpreter must sign the declaration below.*)

Signed at (*place*) on (*date*) 2014.

.....
Signature of investigator

.....
Signature of witness

Declaration by interpreter

I (*name*) declare that:

- I assisted the investigator (*name*) to explain the information in this document to (*name of participant*) using the language medium Tswana.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) on (*date*)201

.....
Signature of interpreter

.....
Signature of witness

ADDENDUM C: TSWANA CONSENT FORM

Nr:

PAMPIRI TSHEDIMOSETSO YA MOTSAJA KAROLO LE FOROMO YA
TUMALLANO

SETLHOGO SA POROJEKE YA DIPATLISISO:

Ditswaki tsa Mekgato ya Bomme ba ba tshwaeditsweng ke mogare wa HIV ba amogela
Tlhokomelo ya melemo ya *Anti-retroviral* e bile ba tshotse masea pele ga nako

NOMORO ITSHUPO:

S13/09/165

MMATLISISI MOGOLO:

Carike den Boer

ATERESE:

Karolo ya Kotlo ya Botho, Lephata la Melemo le Saentshe ya Boitekanelo Unibesithi ya
Pretoria

012 354 1130

NOMORO YA MOGALA:

072 690 6990

O lalediwa go tsaya karolo mo porojekeng ya patlisiso. Ka kopo tsaya nako go buisa
tshedimosetso e e tlhagisitsweng fa, eo e tlhalosang dintlha tsa porojeke e. Ka kopo botsa
badiri ba patlisiso dipotso dingwe le dingwe ka karolo nngwe le nngwe eo o sa e
tlhaloganyeng sentle. Go botlhokwa thata gore o kgotsofale le go tlhaloganya sentle se
patlisiso e e leng ka ga sone le ka fa o ka amegang ka teng. Gape, botsaya karolo ba gago ke
go **ithaopa gotlhelele** e bile o letleletswe go se dumele go tsaya karolo. Fa e le gore ga o
dumele, seno ga se go ame gotlhelele. O letleletswe go ikgogela morago mo patlisisong nako
nngwe le nngwe, le fa e le gore o ka dumela go tsaya karolo.

Patlisiso eno e tlhomamisitswe ke **Komiti ya “Health Research Ethics”** kwa Unibesithi ya **Stellenbosch** le Sepetlele sa Kalafong. E tlile go tsamaisiwa go ya ka ditaelo tsa maitshwaro a setho le melawana ya Tumallano ya boditšhabatšhaba ya Helsinki, Ditaelo tsa Aforika Borwa tsa “*Good Clinical Practice*” le “*Medical Research Council (MRC) Ethical Guidelines for Research*”.

Patlisiso e e ka ga eng?

Mmatlisisi o tla go kopa tetla ya go leba mo faeleng ya gago ya sepetlele go bona tshedimosetso ya letlamorago la boitekanelo ba gago le maemo a gago a HIV. Tshedimosetso yotlhe e tla nna sephiri. Go fitlhela tshedimosetso ya boitekanelo kotlo ba gago o tlile go kadiwa bokete jwa mmele, boleele le letsogo godimo la gago. Mofetoledi a ka nna teng go thusa mmatlisisi ka tsamaiso. Jaanong, o tla kopiwa go rothetsa bonnye ba mekgato mo setshelong. Sampole e le nngwe ya selekano sa 30ml e tlile go tsewa ka letsatsi la bosupa go tloga ka nako eo o simolotseng go tswa mekgato fa sampole e engwe (le yone ya selekano sa 30ml) e tla tsewa malatsi a le robong go tloga nako eo o simolotseng go tswa mekgato. Go solofetswe gore paloyotlhe ya bomme ba le lekgolo ba tla tsaya karolo mo patlisisong kwa Sepetlele sa Kalafong.

Go tla diragala eng mo go nna fa e le gore ke tsaya karolo?

O tlile go nna le maikarabelo a go neela babatlisisi tshedimosetso e e tshepagalang le boammaruri ka ga wena le boitekanelo ba gago. O tlile go kopiwa go neela babatlisisi sampole e le nngwe ya mekgato mo mabakeng a le mabedi. Gape o tlile go kadiwa le go metiwa.

Mmatlisisi o tla meta le bokete jwa mmele wa lesea la gago, boleele le selekano sa tlhogo kgotsa a di tsaya go tswa mo faeleng ya sepetlele.

Mekgato: Jaaka karolo ya patlisiso, re batla go meta ditswaki dingwe mo mekgatong, tse di jaaka poroteine, sukiri, mafura le dimenerale.

Disampole tseno tsa mekgato di tla tsidifatsiwa pele di diriwa diteko kwa laboratoring. Sampole e tla tsewa mo setshelong se se phepa se se tlhokang ditwatsi.

Tshedimosetso go tswa mo faeleng ya gago ya sepetlele e tlile go dirisiwa mo patlisisong go lebilwe ka fao lesea la gago le neng la tsalwa ka teng, maemo a gago a HIV le melemo eo o e

fitlhetseng ka nako ya pelego ya gago. Go tla tsewa basadi botlhe ba ba ithweleng mo Gauteng diteko tsa HIV ka nako ya pelego ka gone go botlhokwa mo go rona go itse maemo a gago a HIV, re tla fitlhela tshedimosetso eo go tswa mo faeleng ya gago ya sepetlele.

Goreng o laleditswe go tsaya karolo?

O tlhophilwe go tsaya karolo mo patlisisong ka gone o tswetse lesea le le tlileng pele ga nako e bile gajaana o le anyisa letsele kgotsa o le fepa ka mekgato.

A maikarabelo a gago e tlile go nna a ma fe?

O tlile go nna le maikarabelo a go neela babatlisisi tshedimosetso e e tshepagalang le boammaruri ka ga wena le boitekanelo jwa gago. O tlile go kopiwa go neela babatlisisi disampole di le pedi tsa mekgato ya gago. O tlile go kadiwa le go metiwa.

A o tlile go ungwa go tsa mo patlisisong eno?

O ka se ungwe go le kalo mo go tseyeng karolo mo patlisisong eno, fela o ka se kgoreletsege ka go se tseye karolo. Tlhokomelo ya boitekanelo ba gago e ka se farologane le ya basadi ba bangwe fa o santse o le kwa sepetlele (ba ba tsayang karolo le ba ba sa tseyeng karolo). Dipelo tsa setlhopha sotlhe di tlile go phasaladiwa kgotsa tsa tlhagisiwa kwa badiri ba bangwe ba boitekanelo, kwa ntle ga go bua leina la gago. Badiri ba bangwe ba boitekanelo (jaaka dingaka, badiri ba manaane a dijo le baoki) ba ka dira patlisiso e e tsweletseng ba dirisa dipelo tsa patlisiso eno, eo mo isagong, e ka nnang le mosola maleba le masea a a fepiwang ka mekgato.

A o go na le dikotsi tse di leng teng mo go tseyeng karolo ga gago mo patlisisong eno?

Nyaa, ga go kotsi epe e e ka go amang.

Ke mang yo a tlileng go bona direkoto tsa boitekanelo ba gago?

Mmatlisisi o tla nna le tetla ya go bona direkoto tsa boitekanelo ba gago . Tshedimosetso yotlhe e tlile go nna bofitlha (sephiri). Dipelo tsa setlhopha sotlhe di tlile go dirisiwa mo lokwalokgang leo le tlileng go phasaladiwa mo jenaleng ya boitekanelo. Leina la gago le ka se dirisiwe-o tlile go nna tlhokaina.

Balaodi ba mmatlisisi le bone ba ka nna le tetla ya go bona tshedimosetso. Tshedimosetso eno, e tlile go nna bofitlha (sephiri).

A botsaya karolo ba me bo tlile go nna sephiri mo patlisisong?

Ee, botsaya karolo ba gago bo tlile go nna sephiri. Disampole tsa mekgato di tlile go nna le dinomoro mme seno ga se reye gore o tlile go senolwa. Ke mmatlisisi a le esi yo o tla itseng gore ke disampole di fe tse e leng tsa ga mme. Mmatlisisi o tlile go baya tshedimosetso yotlhe ya gago sephiri mme ga go tshedimosetso efe e e tla neelwang motho mongwe le mongwe yo o sa direng mo patlisisong kgotsa a amega mo go yone.

A go tla diragala eng fa e le gore go nna le tiragalo ya kgobalo e direga ka gone o tsaya karolo mo tshekatshekong ya patlisiso?

Ga go kgonagalo e e kalo ya gore go ka nna le sengwe se se phoso se se ka diragalang. Fa e le gore ga o kgone go neela mmatlisisi sampole ya mekgato kgotsa ga o kgone go emelela gore o kadiwe, mmatlisisi o tla go kopa gape mo nakong e e tlang.

A o tlile go duelwa go tsaya karolo mo patlisisong eno, a go na le ditshenyegelo tse di leng teng?

Nnyaa, o ka se ke wa duelwa go tsaya karolo mo patlisisong . Ga go na dituelo tse di go amang, fa e le gore o tsaya karolo.

A go na le sengwe se o tshwanetsweng ke go se itse kgotsa go se dira?

O ka ikgolaganya le Komiti ya “*Health Research Ethics*” mo go 012-938 9207 fa e le gore o na le matshwenego kgotsa dingongorego tse di sa tlhagisiwang ke mmatlisisi.

O ka leletsa le mmatlisisi mogolo, Carike den Boer, nako nngwe le nngwe mo go 072 690 6990 fa e le gore o na le dipotso maleba le patlisiso.

O tla fitlhela khopi ya tshedimosetso eno le foromo ya tumallano gore o kgone go di baya jaaka rekoto ya gago.

Maikano a motsaya karolo

Ka go saena fa tlase, Nna..... ke dumela go tsaya karolo mo patlisisong ya setlhogo

Ditswaki tsa Mekgato ya Bomme ba ba tshwaeditsweng ke mogare wa HIV ba Amogela Tlhokomelo ya melemo ya *Anti-retroviral* e bile ba tshotse Masea pele ga Nako.

Ke dumela gore:

- Ke buisitse kgotsa ba mpuiseditse tshedimosetso eno le foromo ya tumallano mme e kwadilwe ka puo e ke itseng le go e tlhaloganya.
- Ke nnile le tšhono ya go botsa dipotso mme di arabilwe sentle tsotlhe.
- Ke tlhaloganya gore go tsaya karolo ga me mo patlisisong eno go ka boithapo ba me e bile ga ka gatelelwa go tsaya karolo.
- Nka itlhophele go tlogela patlisiso nako nngwe le nngwe mme ka se otlhaiwe le go otlhaelelwa pele ka mokgwa mongwe le mongwe.
- Nka kopa go tlogela patlisiso pele e wetsiwa, fa e le gore ngaka ya tlhatlhobo kgotsa mmatlisisi o akanya fa go le maleba go ya ka dikgatlhego tsa me, kgotsa fa e le gore ga ke sale morago thulaganyo ya patlisiso, jaaka go dumellanwe.

E saenetswe kwa (*lefelo*).....ka di(*letlha*).....2014.

.....

Mosaeno wa motsaya karolo

.....

Mosaeno wa Paki

Maikano a mmatlisisi

Nna (*leina*).....ke dumela gore:

- Ke tlhalositse tshedimosetso e e mo pampiring go
- Ke mo rotloeditse go botsa dipotso le go tsaya nako sentle go di araba.
- Ke kgotsofetse gore o tlhaloganya sentle dintlha tsotlhe tsa patlisiso, jaaka e tlhagisitswe fa godimo.
- Ke dirisitse / ga ka dirisa mofetoledi. (Fa e le gore mofetoledi o dirisitswe o tshwanetse go saena tumallano e e fa tlase).

Saenetswe kwa(*lefelo*).....ka di(*letlha*).....2014.

.....

Mosaeno wa mmatlisisi

Mosaeno wa Paki

Maikano a mofetoledi

Nna(*leina*) ke dumela gore:

- Ke thusitse mmatlisisi(*leina*) go tlhalosa tshedimosetso mo pampiring eno kwa go (*leina la motsaya karolo*) ke dirisa puo ya Setswana.
- Re mo rotloeditse go botsa dipotso le go tsaya nako sentle go di araba.
- Ke buile sentle dintlha tse di nepagetseng go ya ka fa di tlhagisitsweng mo go nna.
- Ke kgotsofetse gore motsaya karolo o tlhaloganya ka botlalo diteng tsa pampiri ya tumallano e bile o kgotsofaletse dikarabo tsotlhe tsa dipotso tsa gagwe.

Saenetswe kwa(*lefelo*).....ka di (*letlha*).....2014.

.....

Mosaeno wa mofetoledi

Mosaeno wa Paki

ADDENDUM D: AFRIKAANS CONSENT FORM

Nr:

DEELNAME INLIGTINGS PAMFLET EN TOESTEMMINGSVORM

TITEL VAN DIE NAVORSINGSPROJEK:

Borsmelksamestelling van HIV Positiewe Moeders wat, Anti-retrovirale Terapie Ontvang Geboorte geskenk het aan Premature Babas

VERWYSINGSNOMMER:

S13/09/165

PRIMÊRE ONDERSOEKER:

Carike den Boer

ADRES:

Navorsingsetiese Komitee, Fakulteit Gesondheidswetenskappe, Universiteit van Pretoria

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KONTAKNOMMER:

072 690 6990 (Carike den Boer)

U word uitgenooi om aan 'n navorsingstudie deel te neem. Neem asseblief u tyd om die inligting wat die studie beskryf noukeurig deur te lees. Voel vry om die navorsers enige vrae te vra wat onduidelik is of wat u nie verstaan nie. Dit is baie belangrik dat u ten volle tevrede is met die voorwaardes en dat u die prosedures duidelik verstaan. Wees bewus dat u deelname **total vrywillig** is en u mag 'nee' sê om deel te neem.. Indien u weier om deel te neem, sal u nie op enige manier negatief beïnvloed word nie. U kan ook op enige stadium van die studie besluit om u deelname te stop, al het u toestemming gegee om deel te neem.

Die studie was deur die **Gesondheidsnavorsing Etiese Komitee te Universiteit van Stellenbosch** goedgekeur asook deur Kalafong Hospitaal en sal volgens die etiese riglyne en beginsels van die internasionale Deklasie van Helinski, Suid Afrikaanse Riglyne vir Goeie Praktyk en die Mediese Navorsingsraad (MRC) Etiese Riglyne vir Navorsing.

Waaroor handel die studie?

Die navorser sal u vra om in u hospitaallêer te kyk om inligting aangaande u mediese geskiedenis en u HIV status te kry. Alle inligting sal vertroulik en privaat gehou word. Om inligting aangaande u voedingstatus te kry, sal ons ook u gewig, lengte en middelarmomtrek neem. 'n Vertaler mag

teenwoordig wees indien dit sou nodig wees. U sal dan gevra word om een monster van u borsmelk te voorsien: een monsters van 30ml elk op dag 7 vandat u begin borsmelk produseer het en een monster (ook 30ml elk) op dag 9 vandat u begin borsmelk produseer het. Daar word verwag dat daar in total 'n 100 moeders aan die studie sal deelneem by Kalafong Hospitaal.

Wat sal gebeur indien ek besluit om deel te neem?

U sal verantwoordelik wees om vir die navoring eerlike en akkurate inligting aangaande uself, u gesondheid en u dieet te gee. U sal ook gevra word om twee borsmelkmonsters te gee en om geweeg en gemeet te word.

Die navorser sal ook u baba se gewig, lengte en kopomtrek neem of die inligting uit die hospitaallêer kry.

Borsmelk: Deel van die studie behels dat ons sekere bestanddele van u borsmelk soos die proteïen-, suiker-, vet- en mineraalinhoud, meet. Ons sal op dag 7 en dag 8 vandat u begin borsmelk produseer het u vra om 'n borsmelkmonster vir ons te gee.

Hierdie melkmonsters sal gevries word en dan in 'n laboratorium getoets word. Die monster sal in steriele (kiemvrye) houers gehou word.

Inligting aangaande u hospitaallêer sal in die studie gebruik word. Dit sluit in hoe u baba gebore is, u HIV status en die medikasie wat u gedurende u swangerskap ontvang het in. Alle swanger vroue in Gauteng word vir HIV getoets. Omdat dit vir ons belangrik is om u HIV status te weet, sal ons dit in u hospitaallêer kry.

Hoekom is u uitgenooi om deel te neem?

U is gekies om deel te neem, omdat u aan preterm baba geboorte gegee het en op die oomblik borsvoed of borsmelk aan u baba verskaf.

Wat sal u verantwoordelikhede wees?

U sal verantwoordelik wees om eerlike en akkurate inligting aan die navorser aangaande u self en u gesondheid te voorsien. Verder sal die navorser van u verwag om twee monsters van u borsmelk te voorsien asook om geweeg en gemeet te word.

Sal u bevoordeel word deur u deelname?

U sal nie direk bevoordeel word deur deel te neem nie, maar u sal ook nie benadeel word deur te weier om deel te neem nie. U mediese sorg tydens u verblyf in die hospital sal nie van ander vroue wat nie aan die studie deelneem verskil nie. Die resultate van die groep as geheel sal gepubliseer of voorgedra word. Ander professionele gesondheidswerkers (soos dokters, dieetkundiges en verpleegsters) kan die resultate van hierdie studie gebruik om verdere navorsing te doen wat in die toekoms voordele vir babas wat geborsvoed word mag inhou.

Is daar enige risiko's indien ek besluit om deel te neem in die studie?

Nee, u het nie 'n risiko om skade te ly nie.

Wie sal toegang tot my mediese rekords hê?

Die navorser sal toegang tot u mediese rekords hê. Alle inligting sal vertroulik (geheim) gehou word. Die resultate van die groep as geheel sal in 'n artikel gebruik word wat in 'n mediese joernaal gepubliseer sal word. U naam sal nie gebruik word nie - u sal **anoniem** bly.

Die navorser se toesighouers sal toegang tot die inligting verkry. Die inligting wat hulle sien sal konfidensieel (geheim) gehou word en hulle sal nie verdere toegang tot hierdie inligting verkry nie.

Sal my deelname in die studie vertroulik gehou word?

Ja, u deelname sal vertroulik gehou word die borsmelkmonsters sal vir analise en gevolgtrekking gehou word en dan weggegooi word wanneer die studie verby is. Die monsters sal genommer word en kan nie terug na u toe gelei word nie. Slegs die navorser sal weet watter monsters aan watter moeder behoort. Die navorser sal alle inligting streng vertroulik hou en geen inligting sal oor u bekend gemaak word deur iemand wat nie direk by die studie betrokke is nie.

Wat sal gebeur in die onwaarskynlikheid dat u 'n vorm van besering opdoen as 'n direkte gevolg van u deelname in die studie?

Dit is baie onwaarskynlik dat iets sal verkeerd gaan. Indien u nie 'n borsmelkmonster kan voorsien of beweeglik is teen die tyd wat die navorser u daarvoor vra nie, kan u dit op 'n latere stadium doen.

Wat sal u betaal word om deel te neem in die studie en is daar enige kostes betrokke?

Nee, u sal nie vergoed word om in die studie deel te neem nie. U sal ook geen geldelike uitgawes he nie. Is daar enige iets anders wat u moet weet?

U kan die Gesondheidswettenskappe Navorsingskomitee by 021-938 9207 bel indien u navrae of klagtes het wat nie voldoende deur die navorser kan beantwoord word nie.

U kan die hoofnavorser, Carike den Boer, ter enige tyd by 072 690 6990 skakel indien u vrae oor die studie het.

U sal 'n kopie van hierdie inligting en toestemmingsvorm vir u eie rekords ontvang.

Verklaring deur die deelnemer:

Deur hieronder te teken, gee ek toestemming om in die studie deel te neem: Premature Borsmelksamestelling van HIV Positiewe Moeders Wat Anti-retrovirale Terapie Ontvang En Geboorte geskenk het Aan Premature Babas.

Ek verklaar dat:

- Ek die inligting en toestemmingsvorm gelees het of iemand dit vir my gelees het en dat dit in 'n taal is waarin ek vlot en gemaklik is.
- Ek was 'n kans gegun om vrae te vra en dit was voldoende beantwoord.
- Ek verstaan dat my deelname **vrywillig** is en ek voel nie gedruk om deel te neem nie.
- Ek mag deelname staak op enige stadium en sal nie gepenariseer word om so te doen nie.
- Ek mag gevra word om die studie te verlaat voordat dit voltooi is indien die dokter of navorser voel dit nie in my beste belange is nie of as ek nie die studieplan, soos ooreengekom, volg nie.

Geteken by (*plek*) op (*datum*) 2014.

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Handtekening van deelnemer

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Handtekening van getuie

Verklaring deur die navorser

Ek (*naam*) verklaar dat:

- Ek die dokument aan verduidelik het.
- Ek haar aangemoedig het om vrae te vra en die tyd gemeen het om dit te beantwoord.
- Ek is tevrede dat sy alle aspekte van die navorsingstudi ten volle verstaan soos hierbo uiteengesit is.
- Ek het/ het nie 'n vertaler gebruik nie. (Indien 'n vertaler gebruik is, moet die vertaler die verklaring hieronder onderteken.)

Geteken by (*plek*) op (*datum*) 2014.

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Handtekening van deelnemer

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Handtekening van getuie

Verklaring deur vertaler:

Ek (*naam*) verklaar dat:

- Ek in die navorser (*naam*) gehelp het om die inligting in hierdie document aan (*naam van deelnemer*) te verduidelik in die taal Tswana.

- Ons het haar aangemoedig het om vrae te vra en die tyd gemeen het om dit te beantwoord.
- Ek het dit on 'n feitelike korrekte weergawe oorgedra soos wat dit aan my oorgedra was/
- Ek is tevrede dat die deelnemer die inhoud van hierdie toestemmingsdokument verstaan en dat al haar vrae voldoende beantwoord is.

Geteken by (*plek*) op (*datum*) 2014.

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Hantekening van vertaler

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Handtekening van getuie

ADDENDUM E: MUAC PERCENTILES

National Health Statistics Reports ■ Number 10 ■ October 22, 2008

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Table 22. Mid-arm circumference in centimeters for females 20 years of age and older by race and ethnicity and age, by mean, standard error of the mean, and selected percentiles: United States 2003–2006

Race and ethnicity and age	Number examined	Mean	Standard error	Percentile								
				5th	10th	15th	25th	50th	75th	85th	90th	95th
All race and ethnicity groups ¹												
Centimeters												
20 years and over	4,195	31.9	0.15	24.3	25.7	26.5	27.9	31.0	35.1	37.4	39.2	42.0
20–29 years	685	30.3	0.30	23.5	24.2	25.1	26.2	29.0	33.1	35.8	37.6	41.4
30–39 years	652	31.9	0.29	24.7	25.8	26.9	27.9	30.7	35.3	37.9	39.5	41.3
40–49 years	773	32.7	0.28	24.9	26.2	27.1	28.7	31.7	35.9	38.2	40.1	42.4
50–59 years	585	32.8	0.23	25.6	26.3	27.4	28.6	32.0	35.9	38.5	40.0	42.3
60–69 years	683	33.0	0.20	25.5	27.0	27.8	29.0	32.1	36.1	37.9	39.5	43.7
70–79 years	452	31.3	0.28	24.4	25.8	26.7	27.9	30.6	34.1	35.9	37.3	39.5
80 years and over	365	29.2	0.23	22.7	23.8	24.9	26.1	28.9	32.0	33.5	34.5	36.0
Non-Hispanic white												
20 years and over	2,158	31.7	0.18	24.2	25.6	26.4	27.8	30.8	34.8	36.9	38.7	41.5
20–39 years	602	30.8	0.30	23.7	24.9	25.7	27.1	29.5	33.5	36.3	38.3	40.6
40–59 years	665	32.5	0.23	25.0	26.1	27.0	28.4	31.6	35.6	37.9	39.8	42.2
60 years and over	891	31.4	0.17	23.8	25.6	26.6	27.9	30.9	34.4	36.3	37.4	40.1
Non-Hispanic black												
20 years and over	925	34.4	0.25	25.3	26.8	28.2	29.9	33.9	38.0	40.5	42.3	45.6
20–39 years	319	33.6	0.38	24.6	26.0	27.1	28.8	33.0	37.5	40.0	41.5	44.6
40–59 years	347	35.3	0.31	26.1	27.8	29.6	31.0	34.7	39.2	41.4	43.1	45.8
60 years and over	259	34.2	0.45	25.7	26.8	28.3	30.1	34.1	37.3	39.7	41.3	45.3
Mexican American												
20 years and over	798	32.2	0.25	25.3	26.4	27.2	28.6	31.6	35.1	37.4	38.6	40.5
20–39 years	294	31.7	0.34	25.0	26.0	26.5	28.0	31.1	34.6	36.9	37.9	40.3
40–59 years	231	33.2	0.29	26.6	27.7	28.3	29.7	32.5	36.0	38.1	39.2	40.5
60 years and over	273	32.1	0.30	25.5	26.2	27.5	28.5	31.4	35.2	36.5	38.3	40.5

ADDENDUM F: FENTON GROWTH CHART

